

ANTICANCER BIANGELOYL SAPONINS

- 5 This application claims benefit of U.S. Serial No. 10/906,303, filed February 14, 2005, Continuation-In-Part application of International Application No. PCT/US04/43465, filed December 23, 2004, which is a Continuation-In-Part application of Int'l App'l No. PCT/US04/33359, filed October 8, 2004, which claims benefit of U.S. Serial Nos. 60/532,101, filed December 23, 2003, and 60/509,851, filed October 9, 2003; and which
- 10 claims benefit of U.S. Serial Nos. 60/617,379, filed October 8, 2004, 60/613,811, filed September 27, 2004, and 60/607,858, filed September 7, 2004. The contents of these preceding applications are hereby incorporated in their entireties by reference into this application.
- 15 Throughout this application, various publications are referenced. Disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

FIELD OF THE INVENTION

- 20 This invention relates to extracts from a plant called Wenguanguo or Xanthoceras sorbifolia, their uses and functions, and methods of their preparation. This invention further relates to novel compounds obtainable from Xanthoceras sorbifolia and plants from the sapindaceae family.

25 BACKGROUND OF THE INVENTION

- Wenguanguo is a species of the sapindaceae family. Its scientific name is Xanthoceras sorbifolia Bunge. Wenguanguo is the common Chinese name. Others are Wenguannguo, Wenguanmu, Wenguanhua, Xilacedeng, Goldenhorn and Yellowhorn. Wenguanguo is grown in Liaoning, Jilin, Hebei, Shandong, Jiangsu, Henan, Shanxi,
- 30 Shaanxi, Gansu, Ningxia and Inner Mongolia, China. Its seeds, leaves and flowers are

edible and have been used as a folk or traditional medicine to treat enuresis for centuries. Its branches and woods are also used as a folk or traditional medicine. For more detailed information and background or relevant art of the present invention, please refer to page 1, lines 25-38, to page 13 of International PCT Application No. PCT/US04/33359, filed October 8, 2004, and U.S. Serial No. 10/906,303, filed February 14, 2005. The contents of these preceding applications are hereby incorporated in their entireties by reference into this application.

Yingjie Chen, Tadahiro Takeda and Yukio Ogihara reported in Chem. Pharm. Bull 33(4)1387-1394(1985) described a study on the constituent of *Xanthoceras sorbifolia* Bunge. See Section V. Saponins from the Fruits of *Xanthoceras sorbifolia* Bunge. Four new saponins were isolated from the fruits of *Xanthoceras sorbifolia* Bunge. The structures of these saponins are bunkankasaponins A, B, C and D. The chemical name of these compounds are:

22-O-acetyl-21-O-(4-O-acetyl-3-O-angeloyl)- β -D-fucopyranosyl-3-O- $[\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 2)- β -D-glucuronopyranosyl] protoaecigenin
22-O-acetyl-21-O-(3,4-di-O-angeloyl) - β -D-fucopyranosyl -3-O- $[\beta$ -D-glucopyranosyl- (1 $\rightarrow$ 2)- β -D-glucuronopyranosyl] protoaecigenin
28-O-acetyl-21-O-(4-O-acetyl-3-O-angeloyl)- β -D-fucopyranosyl-3-O- $[\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 2)- β -D-glucuronopyranosyl] protoaecigenin
28-O-acetyl-21-O-(3,4-di-O-angeloyl) - β -D-fucopyranosyl -3-O- $[\beta$ -D-glucopyranosyl- (1 $\rightarrow$ 2)- β -D-glucuronopyranosyl] protoaecigenin.

The functions of these compounds were not previously disclosed.

Yingjie Chen, Tadahiro Takeda and Yukio Ogihara reported in Chem. Pharm. Bull 33(3)1043-1048(1985) described studies on the constituent of *Xanthoceras sorbifolia* Bunge. See Section IV. Structures of the Miner Prosapogenin. The prosapogenins from the partial hydrolyzate of fruit saponin of *Xanthoceras sorbifolia* were examined, and are characterized as:

16-O-acetyl-21-O-(3,4-di-O-angeloyl- β -D-fucopyranosyl) protoaescigenin
22-O-acetyl-21-O-(3,4-di-O-angeloyl- β -D-fucopyranosyl) protoaescigenin 3-
O- β -D-glucuronopyranoside.

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The functions of these compounds were not previously disclosed.

Yingjie Chen, Tadahiro Takeda and Yukio Ogihara. Chem. Pharm. Bull 33(1)127-
134(1985) described studies on the constituent of *Xanthoceras sorbifolia* Bunge. See
10 Section III. Minor Prosapogenins aponins from the Fruits of *Xanthoceras sorbifolia*
Bunge. The structure of 3 minor prosapogenins, obtained by acid hydrolysis of the
crude saponin faction, were characterized as:

21-O-(3,4-di-O-angeloyl)- β -D-fucopyranosyltheasapogenol B
15 21-O-(4-O-acetyl-3-O-angeloyl)- β -D-fucopyranosyltheasapogenol B
21-O-(4-O-acetyl-3-O-angeloyl)- β -D-fucopyranosyl-22-O-
acetylprotoaescigenin.

The functions of these compounds were not previously disclosed.

20

Yingjie Chen, Tadahiro Takeda and Yukio Ogihara in Chem. Pharm. Bull 33(4)1387-
1394(1985) described a study on the constituent of *Xanthoceras sorbifolia* Bunge. See
Section II. Major Sapogenol and prosapogenin from the Fruits of *Xanthoceras sorbifolia*.
In addition to above studies, saponins with angeloyl groups attached were also reported
25 in the following reports.

Laurence Voutquenne, Cecile Kokougan. Catherine Lavaud, Isabelle Pouny, Marc
Litaudon."Triterpenoid saponins and Acylated prosapogenins from *Harpullia austro-*
caledonica." Phytochemistry 59 (2002) 825-832.

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Zhong Jaing, Jean-francois Gallard, Marie-Therese Adeline, Vincent Dumontet, Mai Van Tri, Thierry Sevenet, and Mary Pais "Six Triterpenoid Saponins from *Maesa laxiflora*." *J. Nat. Prod.* (1999), 62, 873-876.

- 5 Young Seo, John M. Berger, Jennine Hoch, Kim M Neddermann, Isia Bursuker, Steven W. Mamber and David G. Kingston. "A new Triterpene Saponin from *Pittosporum viridiflorum* from the Madagascar Rainforest ". *J. Nat. Prod.* 2002, 65, 65-68.

- Xiu-Wei Yang, Jing Zhao, Xue-Hui Lui, Chao-Mei Ma, Masao Hattori, and Li He Zhang
10 "Anti-HIV-1 Protease Triterpenoid Saponins from the Seeds of *Aesculus chinensis*." *J. Nat. Prod.* (1999), 62, 1510-1513.

- Yi Lu, Tatsuya Umeda, Akihito Yagi, Kanzo Sakata, Tirthankar Chaudhuri, D.K. Ganguly, Secion Sarma. "Triterpenoid Saponins from the roots of the tea plant
15 (*Camellia sinensis* var. *Assamica*)." *Phytochemistry* 53 (2000) 941-946.

- Sandra Apers, Tess E. De Bruyne, Magda Claeys, Arnold J. Viletinck, Luc A.C. Pieters.
"New acylated triterpenoid saponins from *Maesa laceceolata*." *Phytochemistry* 52
(1999) 1121-1131.

- 20 Ilaria D'Acquarica, Maria Cristina, Di Giovanni, Francesco Gasparrini, Domenico Misiti, Claudio D' Arrigo, Nicolina Fagnano, Decimo Guarnieri, Giovanni Iacono, Giuseppe Bifulco and Raffaele Riccio. "Isolation and structure elucidation of four new triterpenoid estersaponins from fruits of the *Pittosporum tobira* AIT." *Tetrahedron* 58 (2002) 10127-
25 10136.

Cancer cells are defined by two heritable properties: (1) they reproduce in defiance of normal restraints on cell division; and (2) they invade and colonize territories normally reserved for other cells.

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Cancers require mutations of one to many genes for its development, and they are classified according to the tissue and cell type from which they arise. Cancers arising from epithelial cells are named carcinomas; those arising from connective tissue or muscle cells are named sarcomas. In addition, there are cancers called leukemias, which are derived from hemopoietic cells. Cancers can also develop from cells of the nervous system.

Cancers originating from different types of cells are, in general, very different diseases. Each cancer has characteristics that reflect its origin. Even when a cancer has metastasized and proliferated out of control, its origins can be traced back to a single, primary tumor. Therefore, it is important to develop drugs or compounds capable of targeting various types of cancer cells.

Ovarian cancer is the 5th leading cause of cancer death in women and the leading cause of death from gynecologic malignancies. In the United States, females have a 1.4 to 2.5%, or 1 out of 40-60 women, lifelong chance of developing ovarian cancer. Older women are at highest risk. More than half of the deaths from ovarian cancer occur in women between 55 and 74 years of age, and approximately one quarter of ovarian cancer deaths occur in women between 35 and 54 years of age. See *MedlinePlus Encyclopedia on ovarian cancer* at <http://www.nlm.nih.gov/medlineplus/ency/article/000889.htm>.

Ovarian cancer is disproportionately deadly for a number of reasons. First, symptoms are vague and non-specific, so women and their physicians frequently attribute them to more common conditions. By the time the cancer is diagnosed, the tumor has often spread beyond the ovaries. Also, ovarian cancers shed malignant cells that frequently implant on the uterus, bladder, bowel, and lining of the bowel wall (omentum). These cells can begin forming new tumor growths before cancer is even suspected. Second, because no cost-effective screening test for ovarian cancer exists, more than 50 percent of women with ovarian cancer are diagnosed in the advanced stages of the disease.

This invention provides compounds or compositions extracted from *Xanthoceras sorbifolia* or plants from the sapindaceae family, or synthesized which have substantial potency against ovarian cancer.

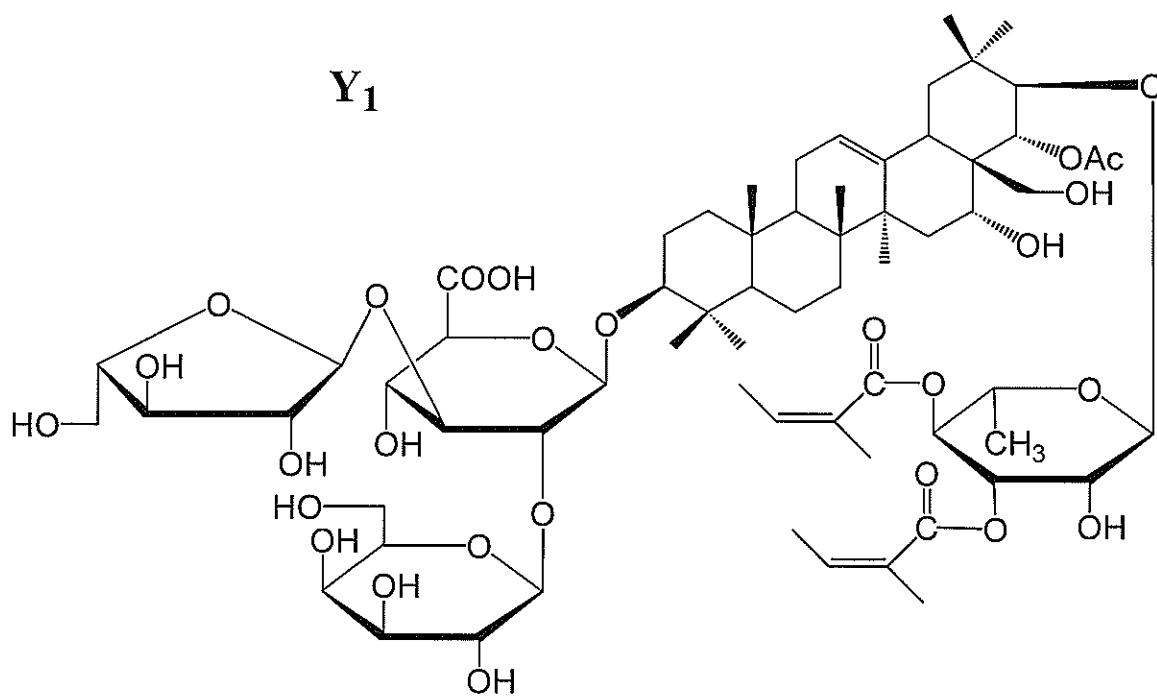
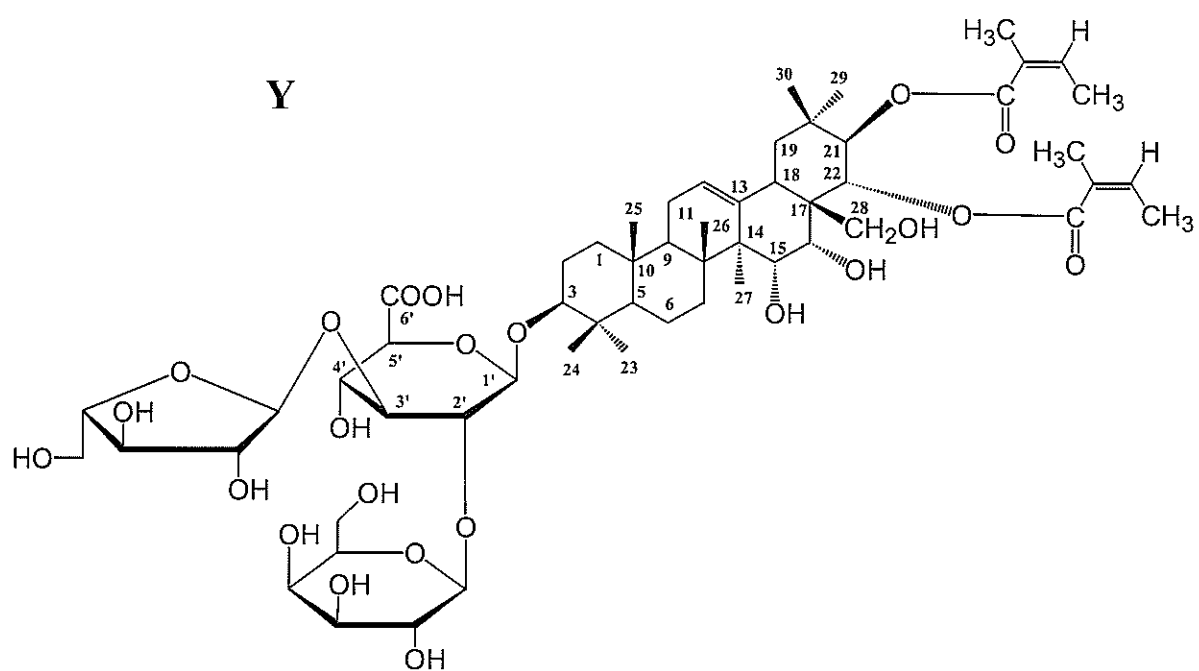
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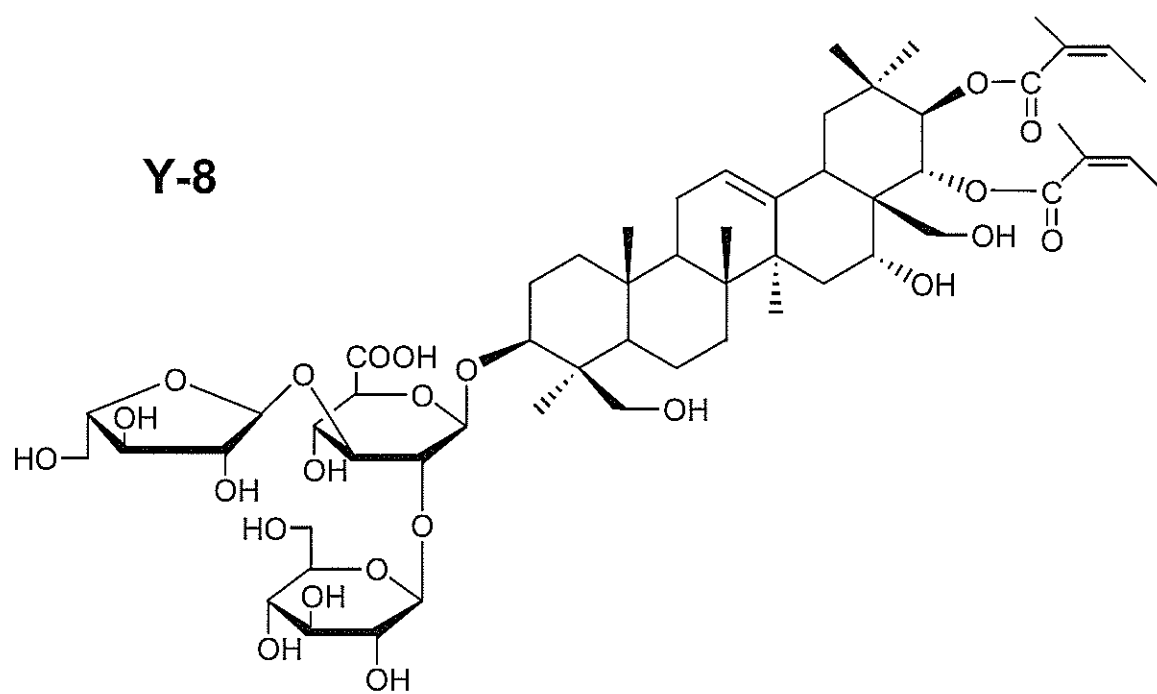
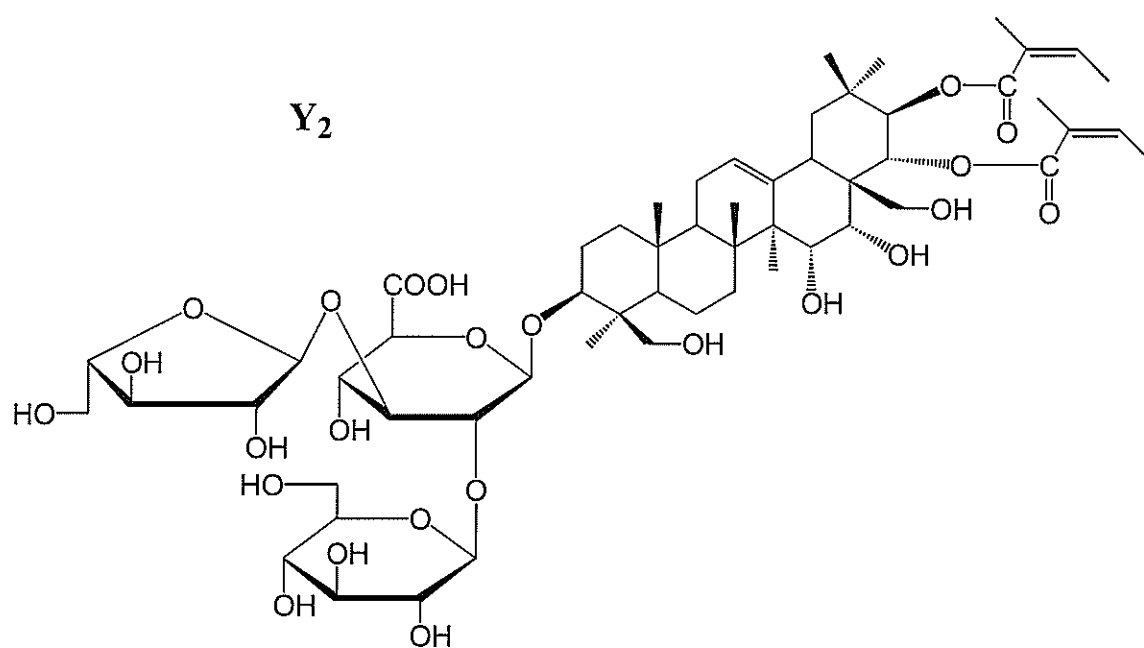
SUMMARY OF THE INVENTION

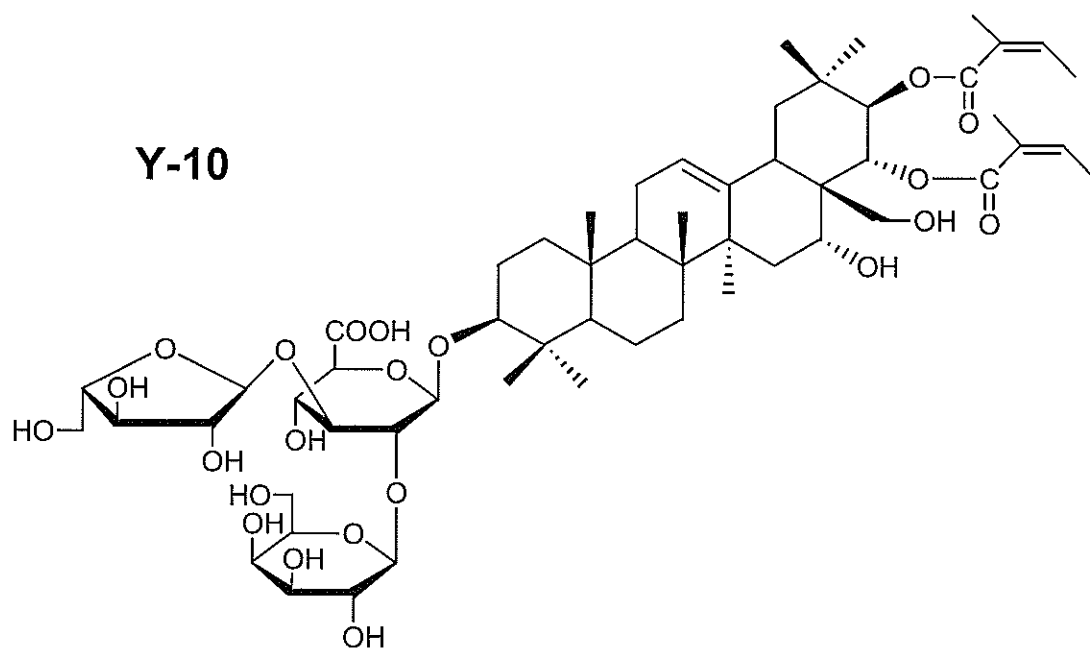
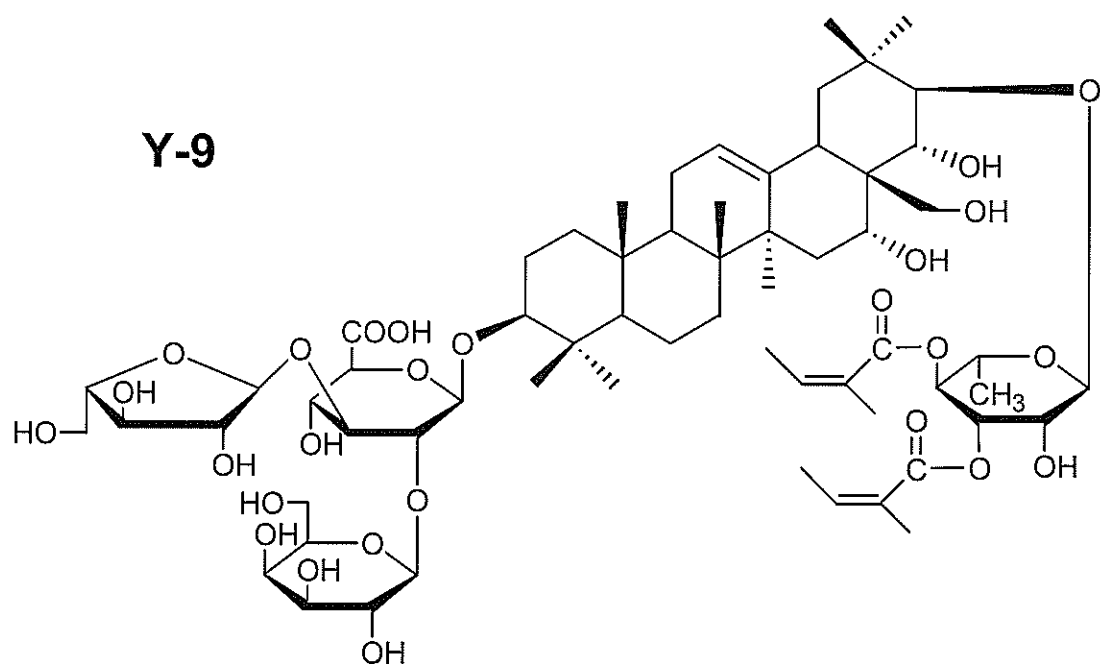
In accordance with these and other objects of the invention, a brief summary of the present invention is presented. Some simplifications and omission may be made in the following summary, which is intended to highlight and introduce some aspects of the present invention, but not to limit its scope. Detailed descriptions of a preferred exemplary embodiment adequate to allow those of ordinary skill in the art to make and use the invention concepts will follow in later sections.

The invention provides six novel compounds of structure (Y1, Y2, Y or Y3, Y8, Y9, Y10) as shown in Figure 1. As used herein, "Y" is also referred to as "Y3".

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The formula, chemical name and common name of these compounds are presented in
 5 Table 1 below.

Table 1. Formula, Chemical Name and Common Name Six Novel Compounds of structure (Y, Y₁, Y₂, Y₈, Y₉, Y₁₀)

Names	Formula	Chemical Name
Y (Y3)	C ₅₇ H ₈₈ O ₂₃	3-O-[β-D-galactopyranosyl(1→2)]-α-L-arabinofuranosyl(1→3)-β-D-glucuronopyranosyl-21,22-O-diangeloyl-3β, 15α, 16α, 21β, 22α, 28-hexahydroxyolean-12-ene,
Y1	C ₆₅ H ₁₀₀ O ₂₇	3-O-[β-D-galactopyranosyl(1→2)]-α-L-arabinofuranosyl(1→3)-β-D-glucuronopyranosyl-21-O-(3,4-diangeloyl)-α-L-rhamnopyranosyl-22-O-acetyl-3β,16α, 21β, 22α, 28-pentahydroxyolean-12-ene
Y2	C ₅₇ H ₈₈ O ₂₄	3-O-[β-D-glucopyranosyl-(1→2)]-α-L-arabinofuranosyl(1→3)-(D-glucuronopyranosyl-21,22-O-diangeloyl-3β, 15α, 16α, 21β, 22α, 24β, 28-heptahydroxyolean-12-ene
Y8	C ₅₇ H ₈₈ O ₂₃	3-O-[β-galactopyranosyl (1→2)]-α-arabinofuranosyl (1→3)-β-glucuronopyranosyl-21, 22-O-diangeloyl-3β, 16α, 21β, 22α, 28-pentahydroxyolean-12-ene
Y9	C ₆₃ H ₉₈ O ₂₆	3-O-[β-galactopyranosyl (1→2)]-α-arabinofuranosyl (1→3)-β-glucuronopyranosyl-21-O-(3,4-diangeloyl)-α-rhamnopyranosyl-3β, 16α, 21β, 22α, 28-pentahydroxyolean-12-ene
Y10	C ₅₇ H ₈₈ O ₂₂	3-O-[β-galactopyranosyl (1→2)]-α-arabinofuranosyl (1→3)-β-glucuronopyranosyl-21, 22-O-diangeloyl-3β, 16α, 21β, 22α, 28-pentahydroxyolean-12-ene

- 5 The above six compounds (Y, Y₁, Y₂, Y₈, Y₉ and Y₁₀) have anti-cancer effect. These compounds inhibit the growth of human ovarian and other cancer cells. See Figure 2, 3 and 4.

10 A consensus sub-structure is identified from these active compounds (Y, Y₁, Y₂, Y₈, Y₉ and Y₁₀). The consensus sub-structure of these compounds is the biangeloyl groups located on adjacent carbons.

For Y, Y₂, Y₈ and Y₁₀, the biangeloyl are located at 21β and 22α of the triterpene backbone. See Figure 5.

For Y1 and Y9, the biangeloyl are located at C3 and C4 of the sugar ring. See Figure 6. Accordingly, the biangeloyl groups of these active compounds (Y, Y1, Y2, Y8, Y9 and Y10) are situated in trans-position in respect to each other on a planar structure. See Figure 7.

Studies of the structure and function relationship of these six structures indicate that changes of the functional groups at C15 and C24 of the triterpene do not affect anticancer activity.

These compounds (Y, Y1, Y2, Y8, Y9 and Y10) are active for inhibition of tumor growth. See Figure 2, 3 and 4. These compounds are purified by methods of chromatography involving FPLC and HPLC as described in Figure 8, 9, 10, 11, 12 and 13.

The compound Y is purified, as shown in Figure 11A, with procedure described in this application. The purified compound Y shows potency ($IC_{50} = 1.5 \text{ ug/ml}$) 10 times higher than the original extract ($IC_{50} = 25 \text{ ug/ml}$) by comparing Figure 2 with Figure 14. Compound Y has a high selectivity toward ovarian cancer. See Figure 15.

The purified compound Y1, Y2, Y8, Y9, and Y10 also show inhibitory activity toward human cancer cells with a higher potency toward ovarian carcinoma. See Figure 3 and 4.

The plant extract containing compound Ys shows inhibitory activity toward the following human cancer cells, i.e., eleven human cancer cell lines were tested in this study, with a higher potency toward ovarian carcinoma. See comparison of activities among these cells in Figures 14, 15 and 16 and Table 3.1. As used herein, Ys or compound Ys is used to denote compound Y or Y3, Y1, Y2, Y8, Y9, Y10 or other compounds obtainable from *Xanthoceras sorbifolia* extract.

This invention provides an extract of *Xanthoceras sorbifolia* capable of inhibiting cancer growth. The cancer includes, but is not limited to ovary cancer, bladder cancer, prostate cancer, leukocytes cancer, and bone cancer.

- 5 The compounds can be isolated from the plant called *Xanthoceras sorbifolia* or can be synthesized chemically, or extracted from other biological sources.

This invention provides a process of producing active compounds from husks, leaves, branches or stems, and fruit-stems, roots and barks of the *Wenguanguo* and can be
10 employed separately or be combined. This invention further discloses methods of their preparations.

In addition to saponin, the extracts contain saccharides, proteins, glycosides, flavonoids, curmarin extracts, alkaloid extracts, organic acid extracts, tannin and others. In this
15 application saponins were investigated and have been shown to possess inhibitory activity against cancer growth.

The compounds or compositions of the present invention may regulate many cellular pathways including the receptors or components of a cell such as G-protein receptor, Fas protein, receptor Tyrosine Kinases, Mitogen, mitogen receptor. The compounds
20 can be isolated from the plant called *Xanthoceras sorbifolia* or can be synthesized chemically, or extracted from other biological sources.

This invention provides compounds, including compound of structures Y, Y1, Y2, Y8, Y9
25 and Y10, obtainable from *Xanthoceras sorbifolia* and capable of inhibiting cancer growth. In an embodiment, the cancer includes, but is not limited to bladder cancer, cervix cancer, prostate cancer, lung cancer, breast cancer, leukocytes cancer, colon cancer, liver cancer, bone cancer, brain cancer, and ovary cancer.

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This invention provides a compound of oleanene triterpenoidal saponin comprising a side chain at Carbon 21 and Carbon 22 of said compound, wherein the side chain comprises angeloyl groups. In an embodiment, the compound comprises one or more sugars, wherein C3 and C4 of the sugar are acylated with angeloyl groups.

5 This invention provides a triterpinoidal saponin compound comprising a triterpene backbone and biangeloyl groups, wherein the biangeloyl groups are attached to 21 β and 22 α of the triterpene backbone, wherein the presence of the biangeloyl group produces anticancer activity.

10 This invention provides a triterpenoidal saponin compound comprising a triterpene backbone and a sugar moiety or sugar chain, wherein the sugar moiety or sugar chain is attached to the triterpene backbone, wherein the sugar moiety or sugar chain further comprises a biangeloyl group, and wherein the presence of the biangeloyl group produces anticancer activity.

15

This invention provides a triterpenoidal saponin compound comprising a triterpene backbone, said triterpene backbone is acylated at either 21 β or 22 α position or at both 21 β and 22 α position with a sugar moiety or sugar chain, wherein at least one sugar in the sugar moiety or sugar chain comprises angeloyl groups attached to the C3 and C4

20

position of said sugar.

As used herein, moiety means one of two or more parts into which something may be divided, such as the various parts of a molecule.

25 In an embodiment, the biangeloyl groups are in the trans-position on a planar structure, and the presence of the biangeloyl group produces anticancer activity.

This invention provides a salt of the above-described compounds.

30 This invention provides a pharmaceutical composition comprising an effective amount of the above-described compounds and a pharmaceutically acceptable carrier(s).

This invention provides a method for isolating compounds from *Xanthoceras sorbifolia* comprising the steps of: extracting *Xanthoceras sorbifolia* powder with an appropriate amount of an organic solvent for an appropriate amount of time to obtain an extract, identifying the bioactive components in the extract; purifying the bioactive components in the extract with FPLC to obtain a fraction of the bioactive component; and isolating the pure bioactive component with preparative HPLC.

This invention provides a compound having a structure verified by NMR spectral data derived from proton NMR, carbon NMR, 2D NMR of the Heteronuclear Multiple Quantum Correlation (HMQC), Heteronuclear Multiple Bond Correlation (HMBC), NOESY and COSY, and Mass spectral data derived from MALDI-TOF and ESI-MS.

This invention provides the chemical features of a compound and its derivatives which are effective against cancer. Due to complexity of nature, the compounds or compositions of the present invention regulate various cellular pathways including but not limiting the followings: the receptors or components such as G-protein receptor, Fas protein, receptor for Tyrosine Kinases, mitogens, or mitogen receptors. TGF Beta-smad, FGF, TGF-beta and TGF-alpha, ras-GTPase-MAP kinase, jun-fos, Src-fyn, Jak-Jnk-STAT, BMP, Wnt, myc-cell proliferation, etc. The *Xanthoceras Sorbifolia* derived compound and/or composition may regulate the components and receptors and re-activates the cell death program.

DETAILED DESCRIPTION OF THE FIGURES

Figure 1 shows the structures of six active anticancer saponins isolated from *Xanthoceras Sorbifolia* extract.

Figure 2 shows the anticancer activity of purified Compound Y. The experiment was performed on ovarian cancer cells (OCAR-3) and the inhibition activity was determined by MTT assay. For details, refer to Experiment 3. Abscissa: Concentration (ug/ml). Ordinate: % Cell Growth. The IC₅₀ is approximately 1- 1.5 ug/ml. A: Point scale. B: Linear scale.

Figure 3 shows the inhibition of the purified Compound Y1 and Compound Y2 on ovarian cancer cells' growth.

Figure 4 shows the anticancer activity of Y, Y8, Y9 and Y10 with ovarian cancer cells determined by MTT assay.

5 **Figure 5** shows the consensus structure derived from four active anticancer saponins (Y, Y2, Y8 and Y10).

Figure 6 shows the consensus structure derived from two active anticancer saponins (Y1 and Y9).

10 **Figure 7** shows a general structural formula derived from the consensus structures of the six active compounds (Y, Y1, Y2, Y8, Y9 and Y10). (A) A consensus active functional group is the biangeloyl group attached to 21 β and 22 α of the triterpene backbone. (B) A consensus active functional group is the biangeloyl group attached at C3 and C4 of a sugar ring (or rhamnose). In both cases, the functional active structure is a biangeloyl group situated in trans-position on a planar structure.

15 **Figure 8** shows the separation of the components of *Xanthoceras sorbifolia* extract by HPLC with a μ bondapak C18 column. Details of experiment were presented in Experiment 2.

20 **Figure 9** shows the elution profile of an extract of *Xanthoceras sorbifolia* in FPLC with 10-80% gradient. Ordinate: Optical density (at 245nm). Abscissa: Fractions (5ml/fraction).

Figure 10 shows the results of the screening of cell growth activity of fractions obtained from FPLC chromatography. The assay was conducted in bladder cells. The fractions obtained from FPLC as shown in Figure 9 were used. As shown in this figure, different components of *Xanthoceras sorbifolia* extracts cause either growth or inhibition effects on cells. Only fraction 5962 (Fraction Y) causes cell inhibition. Fractions 610, 1116 and 1724 cause minor stimulation of cell growth. Abscissa: concentration (μ g/ml). Ordinate: % Cell Growth (determined by MTT assay).

30 **Figure 11** shows HPLC profile of Fraction Y with 45% acetonitrile isocratic elution in a preparative C18 column (Delta Pak C18). Under these conditions, fractions Y (Y3), Y1 and Y2 are well separated from each other and they are subsequently purified. A and B shows the purity of the collected Y3 and Y2 by HPLC under same conditions.

Figure 12 shows the separation profile of Y8-Y10 with 45% acetonitrile isocratic elution in a preparative C18 column (Delta Pak C18).

Figure 13 shows the HPLC profiles of purified Y8, Y9 and Y10.

Figures 14 shows the growth curves of ovarian cancer cells after treatment with the crude extract of *Xanthoceras sorbifolia* as determined by the MTT assay. This study determined the sensitivity of the extract of *Xanthoceras sorbifolia* on cancer cells. In these experiments, cancer cell lines from 11 different human organs were employed. This figure shows that ovary cancer cells are the most sensitive cancer cells in responding to *Xanthoceras Sorbifolia*. Results of other cancer cells were represented in Figures 16A-D. Abscissa: concentration (ug/ml). Ordinate: % Cell Growth (determined by MTT assay).

Figure 15 shows the comparison of potency of Compound Y between ovarian cancer cells and cervical cancer cells. Ovarian cancer cells are much more sensitive than the cervical cancer cells. The IC₅₀ for Compound Y in ovary cells is about 1.5ug/ml while the IC₅₀ in cervical cancer cells is over 20 ug/ml. See also Figure 16D. This result confirms that the activity of Compound Y is selective toward ovary cancer.

Figures 16 A-D show the growth curves of cancer cells derived from different human organs as determined by MTT assay. After treatment with the extract of *Xanthoceras Sorbifolia*, growth curves of different cell lines were presented and their sensitivities (IC₅₀ values) were determined. Sensitivity of cells toward extract can be divided into 4 groups. (1) Most sensitive: ovary cells (presented in Figure 14 and 15). (2): Sensitive: bladder and bone (presented in A). (3) Semi-sensitive: leukocyte and liver (presented in B); prostate, breast and brain (presented in C). (4) Least sensitive: colon, cervix and lung (presented in D). Abscissa: concentration (ug/ml). Ordinate: % Cell Growth (determined by MTT assay).

Figure 17 shows the structure Compound Y with the formula of C₅₇H₈₈O₂₃ and the chemical name of 3-O-[β-D-galactopyranosyl(1→2)]-α-L-arabinofuranosyl(1→3)-β-D-glucuronopyranosyl-21,22-O-diangeloyl-3β, 15α, 16α, 21β, 22α, 28-hexahydroxyolean-12-ene.

Figure 18 shows the spectrum of proton NMR of Compound Y.

Figure 19 shows 2D NMR (HMQC) results of Compound Y.

Figure 20 shows 2D NMR (HMBC) results of Compound Y.

Figure 21 shows the Mass spectrum of compound Y with MALDI-TOF (high mass): Y + Matrix (CHCA) + Angiotensin 1 "two point calibration".

Figure 22 shows the Mass spectrum of compound Y with ESI-MS.

- 5 **Figure 23** shows the structure of Compound Y1 with the formula of $C_{65}H_{100}O_{27}$ and the chemical name of 3-O-[β -D-galactopyranosyl(1 $\rightarrow$ 2)]- α -L-arabinofuranosyl(1 $\rightarrow$ 3)- β -D-glucuronopyranosyl-21-O-(3,4-diangeloyl)- α -L-rhamnophyranosyl-22-O-acetyl-3 β ,16 α , 21 β , 22 α , 28-pentahydroxyolean-12-ene.

Figure 24 shows the Proton NMR spectrum of Compound Y1.

- 10 **Figure 25** shows the 2D NMR (HMQC) results of Compound Y1.

Figure 26 shows the 2D NMR (HMBC) results of Compound Y1.

Figure 27 shows COSY-NMR profile of Compound Y1.

Figure 28 shows the chemical structure and the chemical name of Compound Y2.

Figure 29 shows the proton NMR spectrum of Y2.

- 15 **Figure 30** shows the 2D NMR spectrum of Y2 (HMQC)-level-1.

Figure 31 shows the C13 NMR spectra of compound Y2.

Figure 32 shows the 2D NMR (HMBC)-level-1 spectra of compound Y2.

Figure 33 shows the 2D NMR HOHAHA (TOCSY)-level-1 spectrum of compound Y2.

Figure 34 shows the Mass spectrum of compound Y2 +Matrix + Standards.

- 20 **Figure 35** shows the chemical structure of Y8.

Figure 36 shows H-NMR spectrum of Y8.

Figure 37 shows 2D NMR HMQC (level 1) spectrum of Y8.

Figure 38 shows 2D NMR HMQC (level 2) spectrum of Y8.

Figure 39 shows the chemical structure of Y9.

- 25 **Figure 40** shows H-NMR spectrum of Y9.

Figure 41 shows 2D NMR HMQC (level 1) spectrum of Y9.

Figure 42 shows 2D NMR HMQC (level 2) spectrum of Y9.

Figure 43 shows the chemical structure of Y10.

Figure 44 shows H-NMR spectrum of Y10.

- 30 **Figure 45** shows 2D NMR HMQC (level 1) spectrum of Y10.

Figure 46 shows 2D NMR HMQC (level 2) spectrum of Y10.

Figure 47 shows the chemical structure and the chemical name of Compound R1.

Figure 48 shows the Proton-NMR spectrum of compound R1.

Figure 49 shows the 2D NMR (HMQC) spectrum of compound R1.

Figure 50 shows the 2D NMR (HMBC) spectrum of compound R1.

5 **Figure 51** shows the 2D NMR (COSY) spectrum of compound R1.

Figure 52 shows the C13 NMR spectrum of compound R1.

Figure 53 shows the chemical structure of Compound O54.

Figure 54 shows the Proton-NMR spectra of compound O54.

Figure 55 shows the 2D NMR (HMQC) spectra of compound O54.

10 **Figure 56** shows the 2D NMR (HMBC) spectra of compound O54.

Figure 57 shows the absorption spectrum of *Xanthoceras sorbifolia* extract. Abscissa: Wavelength in nm. Ordinate: Optical Density. The extract has three absorption maximum at 207nm, 278nm and 500nm.

Figure 58 shows the proton NMR spectrum of Y4.

15 **Figure 59** shows the 2D NMR (HMQC) spectrum of Y4.

Figure 60 shows purification of component-R with HPLC. A: Extract from fraction #10 of FPLC (iso-30) was further separated by HPLC. B: Rechromatogram of the major component under same condition as described in A.

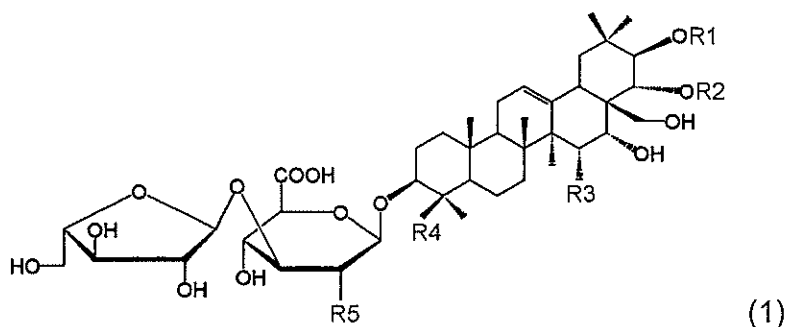
Figure 61. Fractionation of Fraction-O with HPLC with 20% acetonitrile isocratic elution
20 (iso-20).

Figure 62. Rechromatography of O28 and O34 (from iso-20).

Figure 63. Rechromatography of O54 (from iso-20).

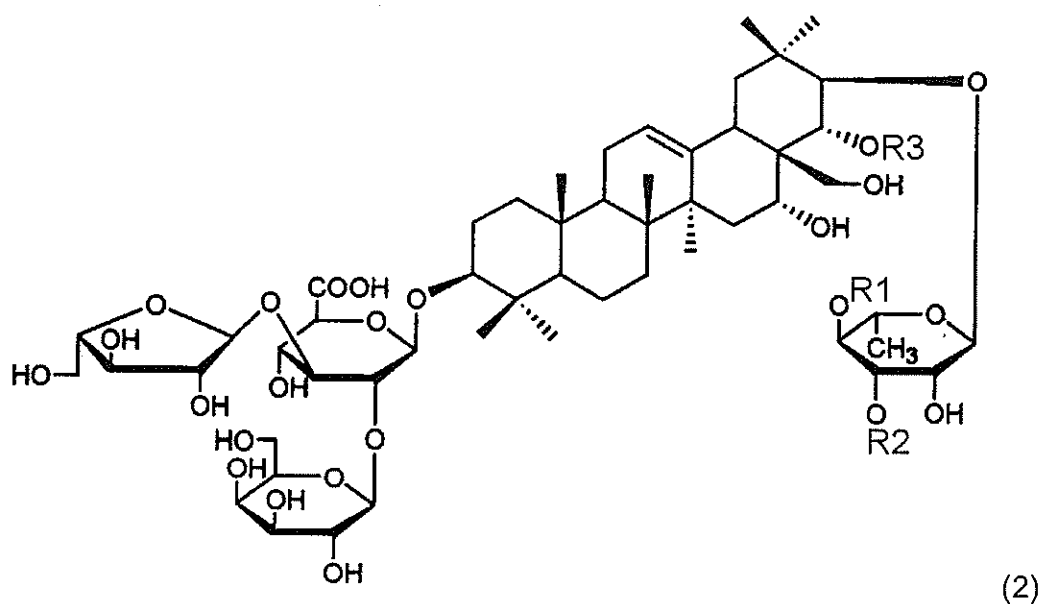
DETAILED DESCRIPTION OF THE INVENTION

25 This invention provides a compound selected from a compound of formula (1):



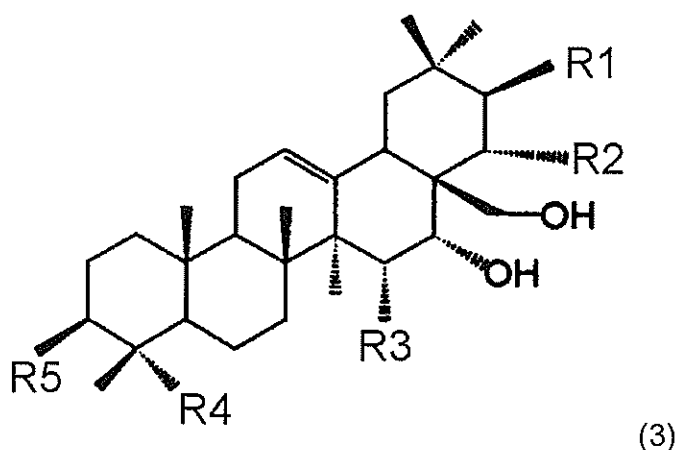
or a salt, ester or derivative thereof, wherein: R1 represents angeloyl group; R2 represents angeloyl group; R3 represents OH or H; R4 represents CH3 or CH2OH; and R5 represents D-glucose or D-Galactose.

- 5 This invention provides a compound selected from a compound of formula (2):



or a salt, ester or derivative thereof, wherein: R1 represents angeloyl group; R2 represents angeloyl group; and R3 represents Ac or H.

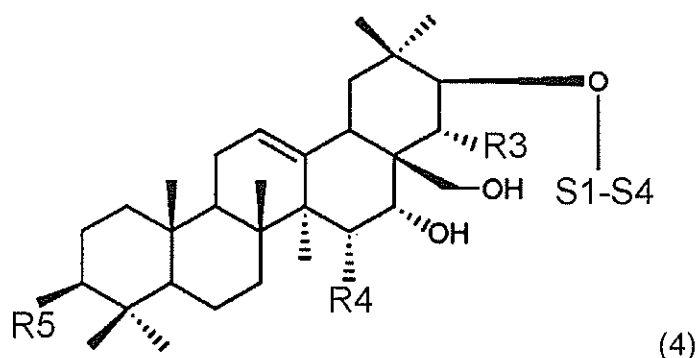
- 10 This invention provides a compound selected from a compound of formula (3):



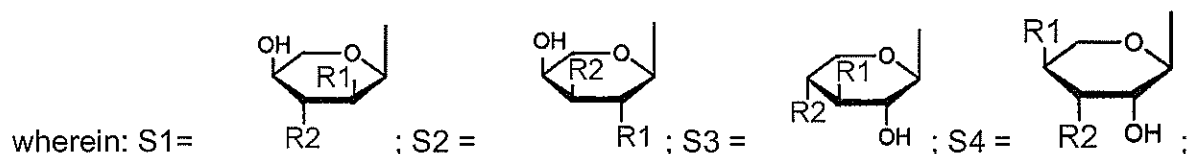
or a salt, ester or derivative thereof, wherein: R1 represents angeloyl group; R2 represents angeloyl group; R3 represents OH or H; R4 represents CH3 or CH2OH; and

R5 represents sugar moiety or sugar chain selected from the group consisting of: D-glucose, D-galactose, L-rhamnose, L-arabinose, D-xylose, alduronic acid, D-glucuronic acid and D-galacturonic acid.

5 This invention provides a compound selected from a compound of formula (4):



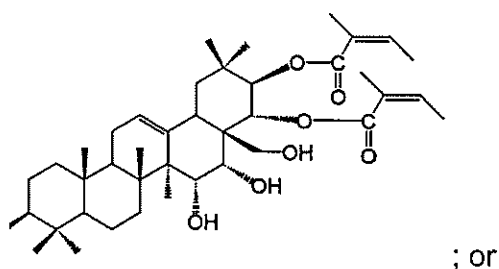
or a salt, ester or derivative thereof,

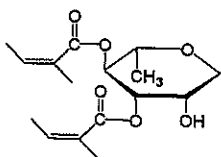


R1 represent angeloyl group; R2 represent angeloyl group; R3 represents Ac or H; R4 represents H or OH; and R5 represents sugar moiety or sugar chain selected from the group consisting of: D-glucose, D-galactose, L-rhamnose, L-arabinose, D-xylose, alduronic acid, D-glucuronic acid and D-galacturonic acid.

In an embodiment, the angeloyl groups are in the trans-position on a planar structure.

15 This invention provides a compound comprising the following structure:





This invention provides a composition for inhibiting tumor cell growth, comprising the above-described compounds. In an embodiment, the composition comprises a suitable carrier. In another embodiment, the composition comprises a pharmaceutically suitable carrier.

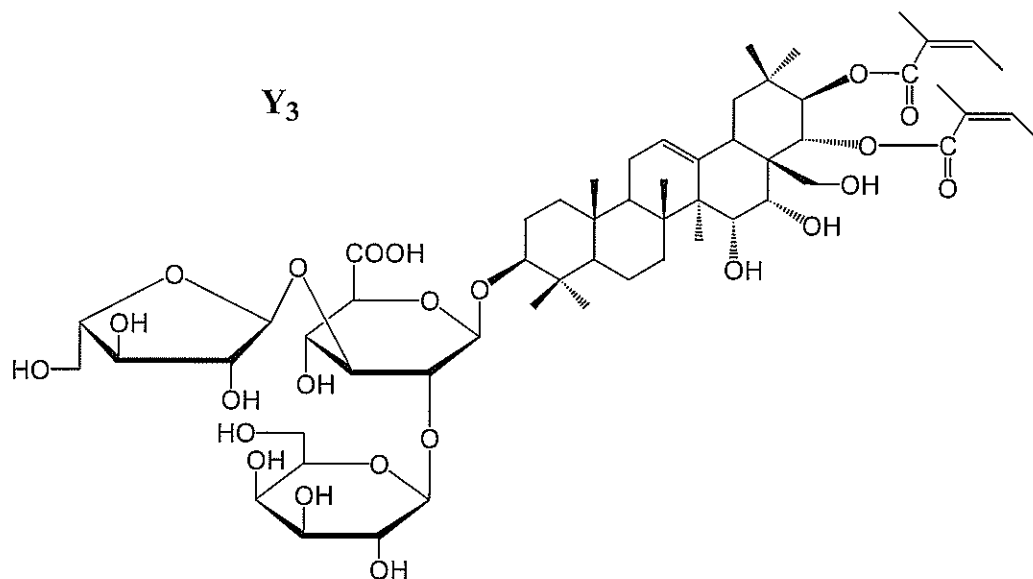
This invention provides a method for treating ovarian cancer in a subject, comprising administering to said subject an effective amount of the above-described compositions.

A method for isolating compounds from *Xanthoceras sorbifolia* herb or plants from the sapindaceae family comprising the steps of: (a) extracting *Xanthoceras sorbifolia* or plant powder with organic solvents to obtain an organic extract; (b) collecting the organic extract; (c) refluxing the organic extract to obtain a second extract; (d) removing the organic solvent from the second extract; (e) drying and sterilizing the second extract to obtain a crude extract powder; (f) fractionating the crude extract powder into components using HPLC and FPLC chromatography with silica gel, C18 and other equivalent solid phase materials; (g) monitoring absorption wavelength at 207nm or 254nm; (h) identifying the bioactive components of the crude extract powder; (i) purifying one or more bioactive components of the crude extract powder with FPLC to obtain one or more fraction of the bioactive component; and (j) isolating the desired fraction of the bioactive component with preparative HPLC.

Compound Y

This invention provides a compound comprising the following structure, i.e., see Figure 17, with the formula of $C_{57}H_{88}O_{23}$ and the name of 3-O- $[\beta$ -D-galactopyranosyl(1 $\rightarrow$ 2)]- α -L-arabinofuranosyl(1 $\rightarrow$ 3)- β -D-glucuronopyranosyl-21,22-O-diangeloyl-3 β , 15 α , 16 α ,

21 β , 22 α , 28-hexahydroxyolean-12-ene, also known as Xanifolia-Y. This compound was isolated from *Xanthoceras sorbifolia*.

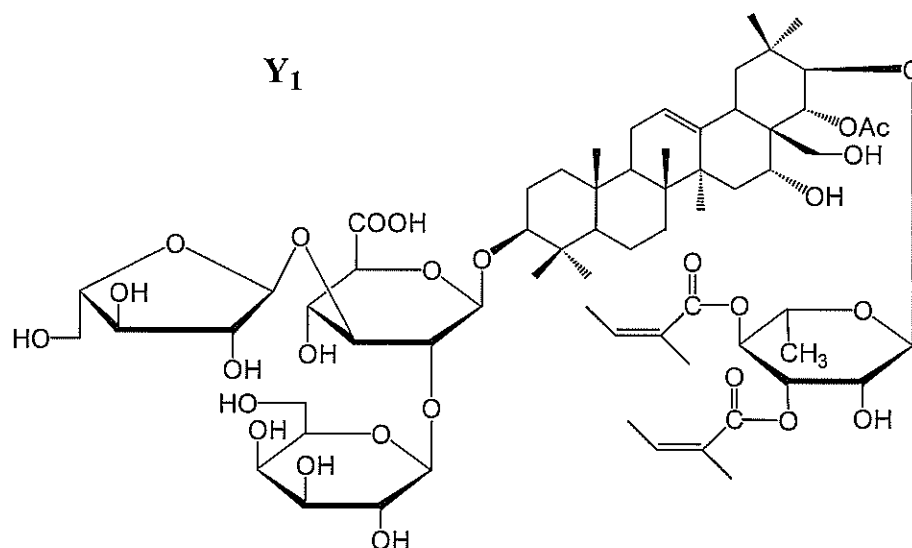


This compound belongs to an oleanene triterpenoidal saponin with a trisaccharide chain attached at C-3 of the aglycone and two angeloyl groups acylated at C-21 and C-22. This compound has anti-cancer activity.

The assignment of this structure is supported by spectral data, i.e., H-NMR, 2D NMR (HMBC, HMQC), and MS (MALDI-TOF, EMS). Accordingly, this compound has the characteristic property as shown in Figures 18-22 or Table 5.1.

Compound Y1

This invention provides another compound comprising the following structure, i.e., see Figure 23, with the formula of C₆₅H₁₀₀O₂₇ and the name of 3-O-[[β -D-galactopyranosyl(1 $\rightarrow$ 2)]- α -L-arabinofuranosyl(1 $\rightarrow$ 3)]- β -D-glucuronopyranosyl-21-O-(3,4-diangeloyl)- α -L-rhamnopyranosyl-22-O-acetyl-3 β ,16 α , 21 β , 22 α , 28-pentahydroxyolean-12-ene, also known as Xanifolia-Y1.

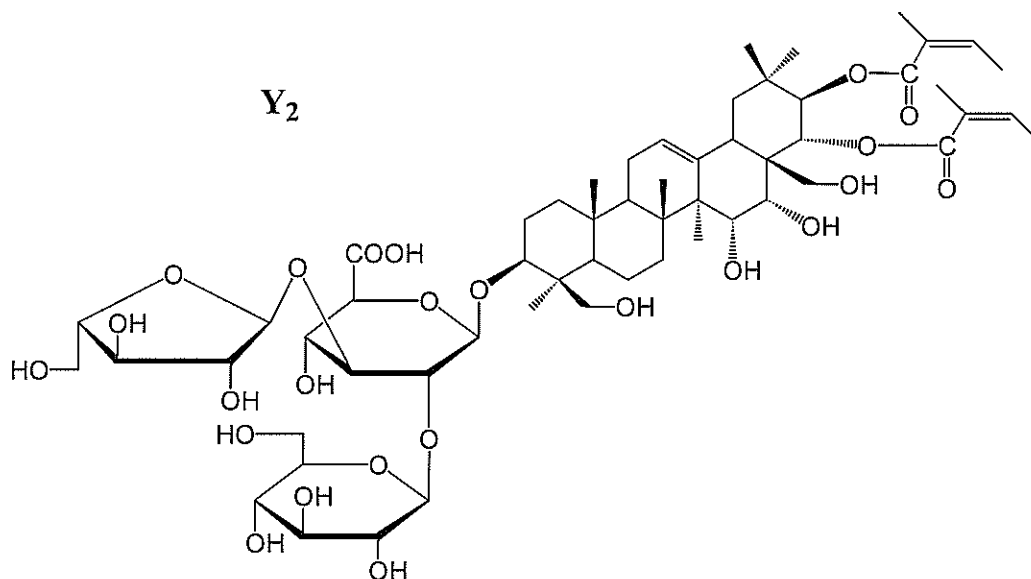


This compound is a bisdesmosidic polyhydroxyoleanene triterpenoidal saponin with a trisaccharide chain at C-3 of the backbone and a monosaccharide moiety at C-21 where two angeloyl groups were acylated at C-3 and C-4 position. This compound has anti-cancer activity.

The assignment of this structure is supported by spectral data, i.e., H-NMR, 2D NMR (HMBC, HMQC, COSY), and MS (MALDI-TOF). Accordingly, this compound has the characteristic property as shown in Figures 24-27.

Compound Y2

This invention provides a third compound comprising the following structure, i.e., see Figure 28, with the formula of $C_{57}H_{88}O_{24}$ and chemical name of 3-O-[β -D-glucopyranosyl-(1 $\rightarrow$ 2)]- α -L-arabinofuranosyl(1 $\rightarrow$ 3)- β -D-glucuronopyranosyl-21,22-O-diangeloyl-3 β , 15 α , 16 α , 21 β , 22 α , 24 β , 28-heptahydroxyolean-12-ene, also known as Y2.

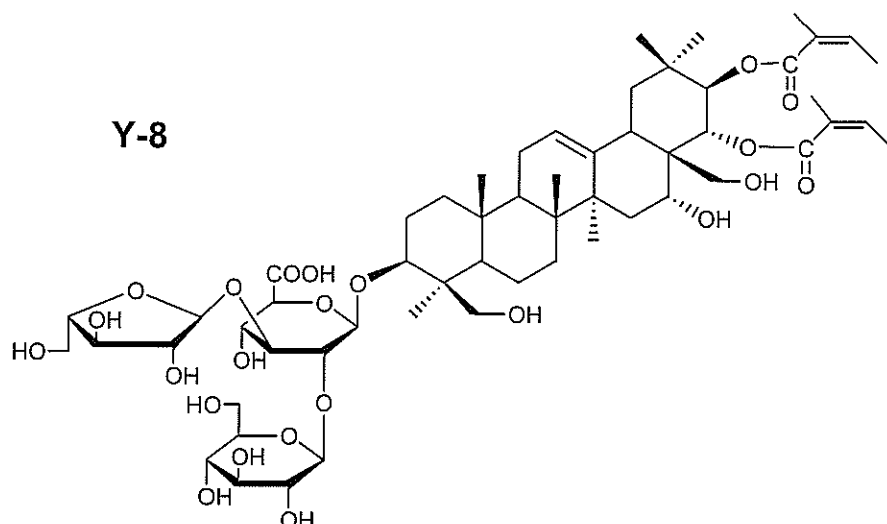


This compound (Y2) belongs to saponins comprising a triterpene, a sugar moiety and angeloyl groups linked to the backbone. The angeloyl groups are linked to the backbone at C21 and C22 positions. This compound has anti-cancer activity.

The assignment of this structure is supported by spectral data, i.e., H-NMR, C-NMR, 2D NMR (HMBC, HMQC, TOCSY), and MS (MALDI-TOF). Accordingly, this compound has the characteristic property as shown in Figures 29-34.

Compound Y8

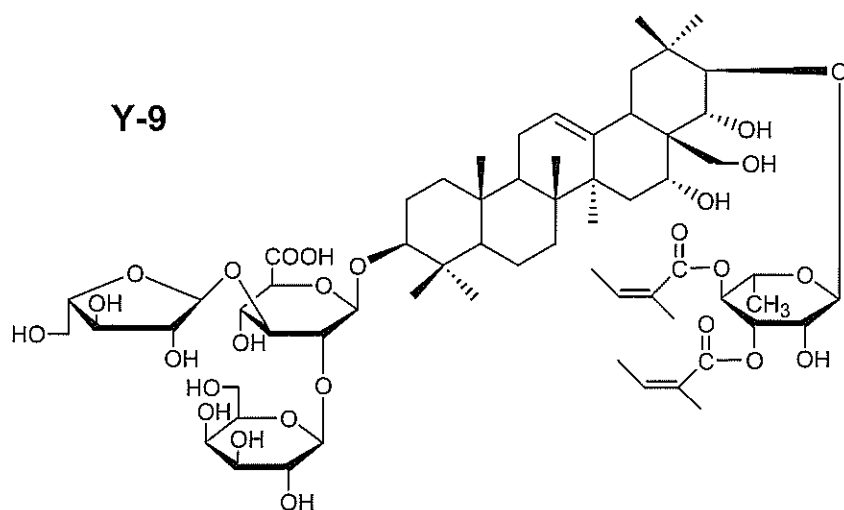
This invention provides a fourth active compound Y8 and the structure was determined by 1D NMR, 2D NMR, and MS analysis. The compound comprises the following structure, i.e. see Figure 35, with the formula of C₅₇H₈₇O₂₃ and chemical name of 3-O- $[\beta$ -glucopyranosyl (1 $\rightarrow$ 2)]- α -arabinofuranosyl (1 $\rightarrow$ 3)- β -glucuronopyranosyl-21, 22-O-diangeloyl-3 β , 16 α , 21 β , 22 α , 24 β , 28-hexahydroxyolean-12-ene.



The assignment of this structure is supported by spectral data, i.e., H-NMR, C13-NMR and 2D NMR (HMQC). Accordingly, this compound has the characteristic property as shown in figures 36-38.

Compound Y9

This invention provides a fifth active compound Y9 and the structure was determined by 1D NMR, 2D NMR, and MS analysis. The compound comprises the following structure, i.e., see Figure 39, with the formula of $C_{63}H_{98}O_{26}$ and chemical name of 3-O-[β -galactopyranosyl (1 $\rightarrow$ 2)]- α -arabinofuranosyl (1 $\rightarrow$ 3)- β -glucuronopyranosyl-21-O-(3,4-diangeloyl)- α -rhamnopyranosyl-3 β , 16 α , 21 β , 22 α , 28-pentahydroxyolean-12-ene.

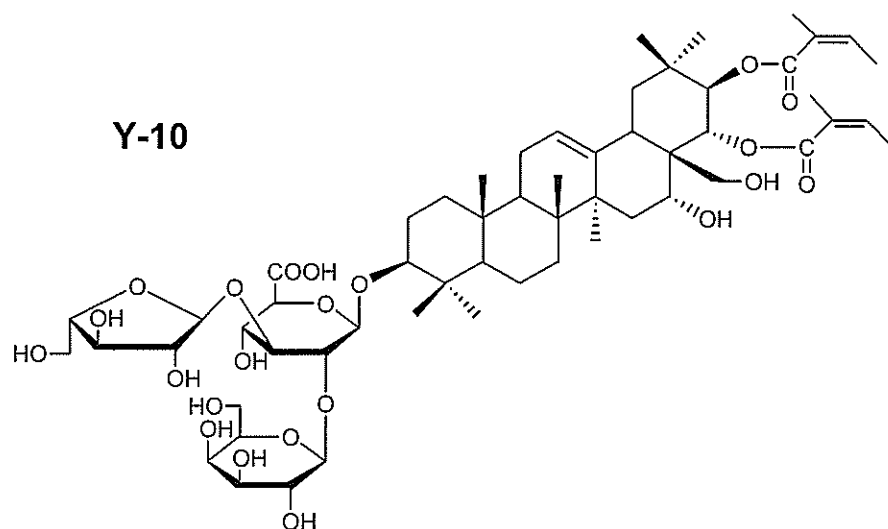


The assignment of this structure is supported by spectral data, i.e., H-NMR, 2D NMR (HMQC and HMBC). Accordingly, this compound has the characteristic property as shown in Figures 40-42.

5

Compound Y10

This invention provides a sixth active compound Y10 and the structure was determined by 1D NMR, 2D NMR and MS analysis. The compound comprises the following structure, i.e., see Figure 43, with the formula of $C_{57}H_{87}O_{22}$ and chemical name of 3-O-
 10 [β-galactopyranosyl (1→2)]-α-arabinofuranosyl (1→3)-β-glucuronopyranosyl-21, 22-O-
 diangeloyl-3β, 16α, 21β, 22α, 28-pentahydroxyolean-12-ene.



The assignment of this structure is supported by spectral data, i.e., H-NMR, C13-NMR and 2D NMR (HMQC). Accordingly, this compound has the characteristic property as shown in figures 44-46.

- 5 This invention provides a compound comprising a sugar and a triterpene or Sapogenin, wherein the triterpene or sapogenin is acylated at Carbon 21 and 22 with Angeloyl groups. In an embodiment, the compound comprises one or more sugars.

This invention shows that extracts of *Xanthoceras sorbifolia* have anticancer activity.

- 10 The experiments for determining the anti-cancer activity employ human cells lines derived from eleven human organs (HTB-9 (bladder), HeLa-S3 (cervix), DU145 (prostate), H460 (lung), MCF-7 (breast), K562 (leukocytes), HCT116 (colon), HepG2 (liver), U2OS (bone), T98G (brain) and OVCAR-3 (ovary)). Among the 11 cell lines studies, their sensitivity toward *Xanthoceras sorbifolia* extract can be divided into four
- 15 groups: (A) most sensitive: Ovary, see Figure 14; (B) Sensitive: bladder, bone, (C) Semi-sensitive: prostate, leukocyte, liver, breast, and brain; and (D) least sensitive: colon, cervix, and lung. See Figure 16A-D. Their IC₅₀ values are listed in Table 3.1.

Table 3.1. IC₅₀ values of *Xanthoceras Sorbifolia* Extract Determined in Different Cancer Cells

Cancer cells from different organs	IC ₅₀ determined by MTT assay (ug/ml)
Ovary (most sensitive)	15-15
Bladder (sensitive)	45-50
Bone	40-55
Prostate (semi-sensitive)	40-50
Leukocyte	45-50
Liver	45-65
Breast	65
Brain	70-85
Colon (least sensitive)	90
Cervix	115

In order to identify the active compounds of *Xanthoceras sorbifolia*, the extracts from *Xanthoceras sorbifolia* were separated by chromatography comprising FPLC (Fast Protein Liquid Chromatography) and HPLC (High Performance Liquid Chromatography).

5 Multiple fractions were obtained by FPLC procedures, i.e., see Figure 9 and HPLC, i.e., see Figure 8. Analysis of the fractions by HPLC shows that the extract comprises 26 identifiable fractions, designated as a to z, which are shown in Figure 8.

Anti-cancer activities of these fractions were determined by the MTT assay. FPLC
10 fraction 5962, i.e., see Figure 10, which corresponding to fraction Y in HPLC, i.e., see Figure 8, has the anti-cancer activity. Fraction 5962 was further separated into 4 components Y1 to Y4, i.e., see Figure 11. Fraction 6365 was further separated into 5-6 components, designated as Y5-Y10. See Figure 12. The compounds Y or Y3, Y1 and Y2 show strong anti-tumor activity, i.e., see Figure 2-3, and were therefore isolated.
15 Similarly, compounds Y8, Y9 and Y10 also show strong anti-tumor activity, i.e., see Figure 4, and were therefore purified. See Figure 13.

The structures of these active compounds, i.e., Y, Y1, Y2, Y8, Y9 and Y10 and their uses are the subject of this application.

20 The inhibition effects of the compounds of the present invention on ovarian cancer cells were evaluated with the MTT assay. Compound Y shows at least 10 times higher potency ($IC_{50} = 1.5 \text{ ug/ml}$), i.e., see Figure 2, than the original crude extract as shown in Figure 14 ($IC_{50} = 20 \text{ ug/ml}$).

25 The selectivity of compound Y toward different cell lines was tested, and it was found that compound Y has a much higher potency toward ovarian cancer cells as compared to the cervical cancer cells. See Figure 15.

This invention provides a method for identifying and isolating the active compounds from plants, herbs or plant extracts. In an embodiment, the extracts include extracts of *Xanthoceras sorbifolia* or of plants from the sapindaceae family.

- 5 This invention provides the chemical structures of six active compounds obtainable from *Xanthoceras sorbifolia* or of plants from the sapindaceae family. The compounds are shown in Figure 1.

10 This invention provides spectral data including H-NMR, C-13-NMR, 2D NMR (HMBC, HMQC, COSY, TOCSY), and MS (MALDI-TOF, ESI-MS) in supporting the assigned structures.

15 This invention provides a consensus sub-structure or functional group from the active compounds purified from fraction Y. The compounds, such as Y or Y3, Y1, Y2, Y8, Y9 and Y10, obtainable from fraction Y are collectively referred to as "Ys". The consensus sub-structure or functional group of these compounds is the biangeloyl groups located on adjacent carbons. For example, in compound Y, Y2, Y8 and Y10, the biangeloyl are located at 21 β and 22 α of the triterpene backbone. See Figure 5. In compound Y1 and Y9, the biangeloyl groups are located at C3 and C4 of the sugar ring. See Figure 6.
20 Accordingly, the biangeloyl groups of these active compounds are situated in trans-position with respect to each other on a planar structure. See Figure 7.

The results of this invention indicate the active functional group of these compounds is a biangeloyl group attached in-trans to adjacent carbons located in a planar structure.
25 See Figure 7.

This invention provides a salt of the above-described compounds.

30 This invention provides a composition comprising the above-described compounds and a suitable carrier.

This invention provides a pharmaceutical composition comprising an effective amount of the above-described compounds and a pharmaceutically acceptable carrier.

5 This invention provides an anti-ovarian cancer agent or composition comprising the above-described compositions.

This invention provides a composition effective against cancer growth. The cancer includes but is not limited to bladder cancer, bone cancer and ovary cancer.

10 This invention provides a composition comprising the above-described compounds and their salts, esters, derivatives or metabolites capable of inhibiting tumour growth.

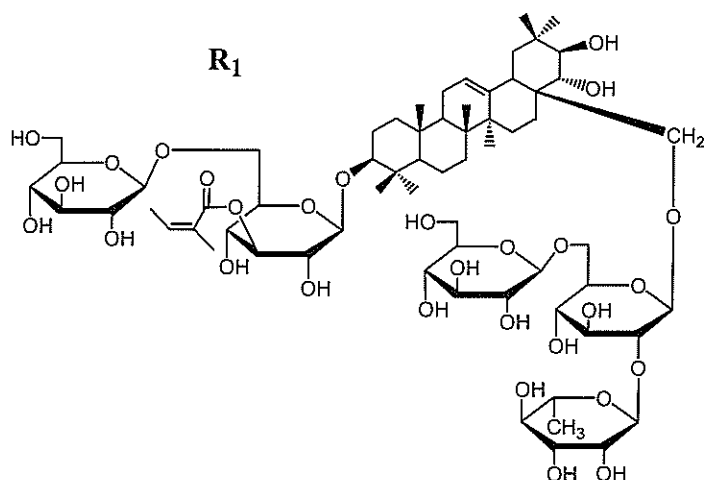
This invention provides a composition comprising the above-described compounds and their salts, esters, derivatives or metabolites capable of inhibiting virus growth and/or
15 activities.

In addition to the compound Ys, other compounds were also purified from fraction R and fraction O of the extract of *Xanthoceras sorbifolia*, which are designated herein as R1 and O54, respectively. Their structures were determined. Preliminary experiments
20 indicate both R1 and O54 do not have anticancer activity.

Compound R1

The structure of Compound R1 shown below and in Figure 47, has a chemical formula of $C_{65}H_{106}O_{29}$ and chemical name of

25 3-O-[angeloyl-(1→3)-β-D-glucopyranosyl-(1→6)]-β-D-glucopyranosyl-28-O-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-3β, 21β, 22α, 28-tetrahydroxyolean-12-ene, also known as Xanifolia-R1.



The assignment of this structure is supported by spectral data, i.e., H-NMR, C-13-NMR, 2D NMR (HMBC, HMQC, COSY), and MS (MALDI-TOF, EMS). Accordingly, this compound has the characteristic property as shown in Figures 48-52.

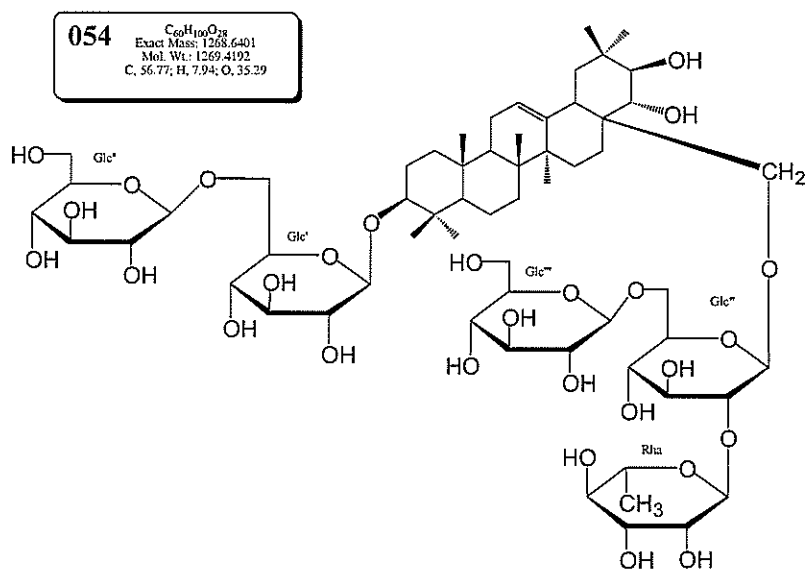
5

Compound-O54

This invention provides a compound O54 purified from the extract of *Xanthoceras sorbifolia*. The structure of O54 was determined and has a formula of C₆₀H₁₀₀O₂₈.

The Structure of Compound O54 is shown below, i.e., see Figure 53:

10



The chemical name of compound-O54 is: 3-O- β -D-glucopyranosyl-(1 $\rightarrow$ 6)]- β -D-glucopyranosyl-28-O-[α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)]- β -D-glucopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranosyl-3 β , 21 β , 22 α , 28-tetrahydroxyolean-12-ene.

- 5 The assignment of this structure is supported by spectral data, i.e., ¹H-NMR, 2D NMR (HMBC, HMQC). Accordingly, this compound has the characteristic property as shown in Figures 54-56.

Summary

- 10 This invention provides methods for identifying and purifying compounds from the plant extract of *Xanthoceras sorbifolia*. Six compounds have been identified and purified, and have been shown to have anticancer activity. These compounds are collectively referred to as triterpenoidal saponins. A consensus sub-structure is identified from these active compounds. A consensus sub-structure or active functional groups of these
15 compounds is the biangeloyl groups located on adjacent carbons. The biangeloyl groups are located at 21 β and 22 α of the triterpene backbone, i.e., see Figure 5, or located at C3 and C4 of the sugar ring, i.e., see Figure 6. Accordingly, the biangeloyl groups of these active compounds are situated in trans-position in respect to each other on a planar structure. See Figure 7. The structures or derivatives of the compounds of
20 the present invention are also obtainable by chemical synthesis or from biological sources.

- This invention will be better understood from examples which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed
25 are merely illustrative of the invention as described more fully in the claims which follow thereafter.

EXPERIMENTAL DETAILS

Experiment 1: Herb Extraction

(a) extracting *Xanthoceras sorbifolia* powder of husks or branches or stems or leaves or kernels or roots or barks with organic solvent at ratio of 1:2 for 4-5 times for 20-35 hours each time to form an organic extract; (b) collecting the organic extract; (c) refluxing the organic extract for 2-3 times at 80°C to form second extracts; (d) removing the organic solvent from the second extract; and (e) drying and sterilizing the extract to form a *Xanthoceras sorbifolia* extract powder.

Experiment 2: Analysis of *Xanthoceras Sorbifolia* Extract Components by HPLC Chromatography

Methods

HPLC. A C-18 reverse phase μ bondapak column (Water P/N 27324) was equilibrated with 10% acetonitrile, 0.005% Trifluoroacetic acid (equilibration solution). An extract of *Xanthoceras sorbifolia* prepared using the methods described in Experiment 1 was dissolved in equilibration solution (1 mg/ml) before applying into the column. 20ug of samples was applied into column. Elution conditions: Fractions were eluted (with flow rate 0.5 ml/min.) with acetonitrile gradient from 10% to 80% in 70 min, and then remains at 80% for 10 min. The acetonitrile concentration then decreased to 10% and remained at 10% for 25 min. The fractions were monitored at 207 nm and recorded in chart with a chart speed of 0.25 cm/min and with OD full scale of 0.128.

Instruments. Waters Model 510 Solvent Delivery System; Waters 484 tunable Absorbance Detector; Waters 745/745B Data Module.

Absorbance analysis. The absorption profile of *Xanthoceras Sorbifolia* extract at various wavelengths was determined. An extract of *Xanthoceras sorbifolia* of the present invention was dissolved in 10% acetonitrile/TFA and scanned at 200-700 nm with a spectrophotometer [Spectronic Ins. Model Gene Sys2].

Results

HPLC. About 60-70 peaks can be accounted for in the profile. Among them four are major peaks, 10 are of medium size and the rest are small fractions. The peaks are labelled with a to z following increased concentration of acetonitrile elution. See Figure 8.

Absorption maximum. Three absorption maximum were identified for *Xanthoceras sorbifolia* plant extract; 207nm, 278nm and 500nm. See Figure 57.

Experiment 3: Determination of the cell-growth activity effected by *Xanthoceras Sorbifolia* Extract with Cancer Cells Derived from Different Human Organs using MTT Assay

Methods and Materials

Cells. Human cancer cell lines were obtained from American Type Culture Collection: HTB-9 (bladder), HeLa-S3 (cervix), DU145 (prostate), H460 (lung), MCF-7 (breast), K562 (leukocytes), HCT116 (colon), HepG2 (liver), U2OS (bone), T98G (brain) and OVCAR-3 (ovary). Cells were grown in culture medium (HeLa-S3, DU145, MCF-7, Hep-G2 and T98G in MEN (Earle's salts); HTB-9, H460, K562, OVCAR-3 in RPMI-1640; HCT-116, U2OS in McCoy-5A) supplemented with 10% fetal calf serum, glutamine and antibiotics in a 5% CO₂ humidified incubator at 37°C.

MTT assay. The procedure for MTT assay followed the method described in (Carmichael et al., 1987) with only minor modifications. Cells were seeded into a 96-wells plate at concentrations of 10,000/well (HTB-9, HeLa, H460, HCT116, T98G, OVCAR-3), 15,000/well (DU145, MCF-7, HepG2, U2OS), or 40,000/well (K562), for 24 hours before drug-treatment. Cells were then exposed to drugs for 48 hours (72 hours for HepG2, U2OS, and 96 hours for MCF-7). After the drug-treatment, MTT (0.5 mg/ml) was added to cultures for an hour. The formation of formazan (product of the reduction of tetrazolium by viable cells) was dissolved with DMSO and the O.D. at 490nm was measured by an ELISA reader [Dynatech. Model MR700]. The MTT level of cells before drug-treatment was also measured (T₀). The % cell-growth (%G) is calculated as:

$$\%G = (TD - T_0 / TC - T_0) \times 100 \quad (1)$$

where TC or TD represent O.D. readings of control or drug-treated cells. When $T_0 > TD$, then the cytotoxicity (LC) expressed as % of the control is calculated as:

$$\%LC = (TD - T_0 / T_0) \times 100. \quad (2)$$

Results

Among the 11 cell lines studies, inhibition of cell-growth after exposure of plant extract was observed. However, their sensitivity toward *Xanthoceras sorbifolia* extract is different. It can be divided into four groups: Most sensitive, i.e., Ovary; Sensitive, i.e., bladder, bone; Semi-sensitive, i.e., prostate, leukocyte, liver, breast, and brain; and Least sensitive, i.e., colon, cervix, and lung. See Figure 14, 15 and 16 A-D. Their IC₅₀ values are listed in Table 3.1.

Table 3.1. IC₅₀ values of *Xanthoceras Sorbifolia* Extract Determined in Different Cancer Cells

Cancer cells from different organs	IC ₅₀ determined by MTT assay (ug/ml)
Ovary (most sensitive)	15-15
Bladder (sensitive)	45-50
Bone	40-55
Prostate (Semi-sensitive)	40-50
Leukocyte	45-50
Liver	45-65
Breast	65
Brain	70-85
Colon (least sensitive)	90
Cervix	115
Lung	110

In addition to cell-growth inhibition, the *Xanthoceras sorbifolia* plant extract also stimulate a minor cell growth at low concentrations in bladder, bone and lung cells. Results indicate that there is a cell or tissue stimulation component(s) in the extract. See Figures 16A and 16D.

5

To investigate the inhibition components of the *Xanthoceras sorbifolia* plant extract, the plant extract was fractionated. Figure 10 shows the results of the screening of fractions obtained after FPLC chromatography for cell growth-inhibition activity. The assay was conducted with bladder cells. The fractions obtained from FPLC, as shown in Figure 9, were used. As shown in Figure 9, different components of *Xanthoceras sorbifolia* extracts cause either growth or inhibition effects on cells. Only fractions 5962, designated as Fraction Y, cause cell growth inhibition. Abscissa: concentration (ug/ml). Ordinate: % Cell Growth (determined by MTT assay).

10

15 **Experiment 4: Purification of the Inhibition Components in the *Xanthoceras Sorbifolia* Extract.**

(A) Fractionation of plant extracts with FPLC

20 **Methods**

Column. Octadecyl functionalized silica gel. Column dimension: 2cm x 28cm; equilibrated with 10% acetonitrile – 0.005% TFA before use.

Sample loading: 1-2 ml, concentration: 100mg/ml in 10% acetonitrile/TFA.

Gradient elution condition: 10-80% acetonitrile in a total volume of 500 ml.

25 Monitor absorption wavelength: at 254nm.

Fraction Collector: 5 ml/fractions (collect from 10% to 72% acetonitrile)

Instrument: AKTA-FPLC, P920 pump; Monitor UPC-900; Frac-900.

Results

30 The elution profile of the chromatography shows 4-5 broad fractions. See Figure 9. These fractions were analyzed with HPLC. Specific components, corresponding to a-z

as specified in Figure 8, are then assigned in these FPLC fractions. FPLC fractions are then grouped into 7 pools and analyzed for cell growth activity in bladder cells with MTT assay. See Experiment 3. It was found that only pool #5962, corresponding to fraction Y in HPLC, contains inhibition activity. See Figure 10. It was also found in later experiments that fractions beyond 62 also show inhibition activity. The components isolated from fractions 63-65 showed inhibition activities. See Figure 4, 12 and 13.

(B) Isolation of Component Ys with Preparative HPLC

10 Methods

Column: A preparative HPLC column (Waters Delta Pak C18-300A);

Elution conditions: 45% acetonitrile isocratic elution with flow rate of 1 ml/min.

Fractions are monitored at 207nm and were collected and lyophilized.

15 Results

Final separation of Y fractions was achieved by HPLC with a preparative column. See Figure 11 and 12. These fractions, which include compound Y1, Y2, Y or Y3 and Y4, were collected. Re-chromatography of compound Y showed a single peak in HPLC with a C18 reverse phase column. See Figure 11A and 11B. Re-chromatography of the compound Y8, Y9 and Y10 showed a single peak in HPLC with a C18 reverse phase column. See Figure 13.

(C) Appearance and solubility

The pure compound Ys is an amorphous white powder, soluble in aqueous alcohol, i.e., methanol or ethanol, 50% acetonitrile and 100% pyridine.

(D) Inhibition analysis of Compound Ys with MTT assay

Inhibition analysis of compound Y was determined with MTT assay. Figure 2 shows that compound Y has activity against ovarian cancer cells (OCAR-3) with IC50 value of

1.5 ug/ml which is 10-15 times more potent than the unpurified extract shown in Figure 14.

Figure 15 shows the selectivity of compound Y to ovarian cancer cells compared with cervical cancer cells (HeLa). Figure 3 shows the inhibition activities of compound Y1 and Y2 on the growth of ovarian cancer cells (OCAR-3). Figure 4 shows the inhibition activities of compound Y, Y8, Y9 and Y10 on the growth of ovarian cancer cells (OCAR-3).

Experiment 5: Determination of the Chemical Structure

Methods

NMR analysis. The pure compound Y of *Xanthoceras sorbifolia* was dissolved in pyridine-D5 with 0.05% v/v TMS. All NMR spectra were acquired using a Bruker Avance 600 MHz NMR spectrometer with a QXI probe ($^1\text{H}/^{13}\text{C}/^{15}\text{N}/^{31}\text{P}$) at 298 K. The numbers of scans for 1D ^1H spectra were 16 to 128, depending on the sample concentration. 2D HMQC spectra were recorded with spectral widths of $6000 \times 24,000$ Hz and data points of 2024×256 for t_2 and t_1 dimensions, respectively. The number of scans were 4 to 128. 2D HMBC were acquired with spectral widths of $6000 \times 30,000$ Hz and data points of 2024×512 for t_2 and t_1 dimensions, respectively. The numbers of scans were 64. The 2D data were zero-filled in t_1 dimension to double the data points, multiplied by cosine-square-bell window functions in both t_1 and t_2 dimensions, and Fourier-transformed using software XWIN-NMR. The final real matrix sizes of these 2D spectra are 2048×256 and 2048×512 data points ($F_2 \times F_1$) for HMQC and HMBC, respectively.

Mass spectral analysis. The mass of samples was analyzed by (A) MALDI-TOF Mass Spectrometry and by (B) ESI-MS Mass spectrometry. (A) Samples for MALDI-TOF were first dissolved in acetonitrile, and then mixed with the matrix CHCA, i.e., Alpha-cyano-4-hydroxycinnamic acid, 10mg CHCA/mL in 50:50 water/acetonitrile and 0.1% TFA in final concentration. The molecular weight was determined by the high resolution

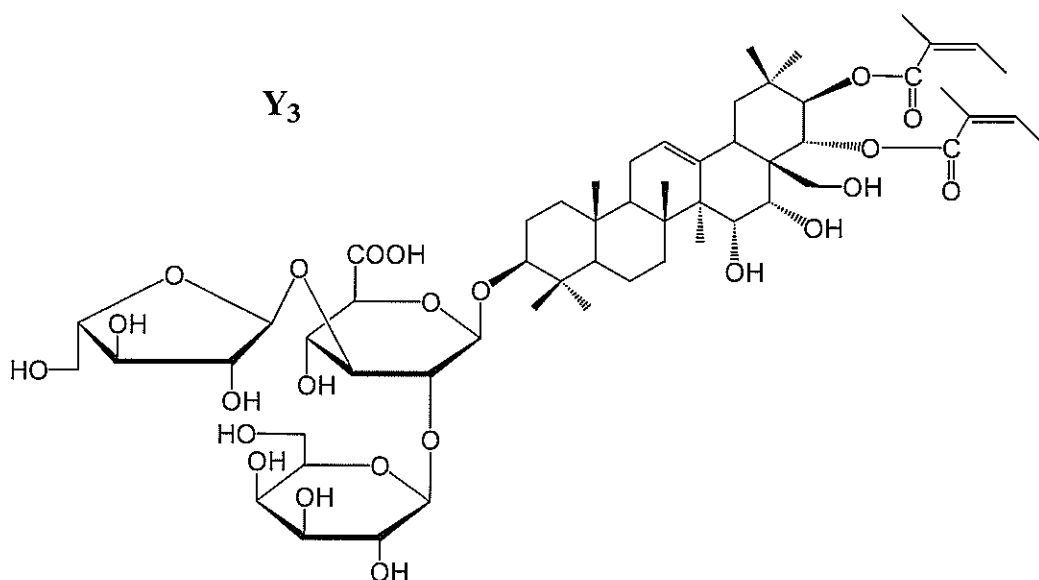
mass spectroscopy analysis with standards. (B) For ESI, the sample was analyzed with LCQ DECA XP Plus machine made by Thermo Finnigan. It is ionized with ESI source and the solvent for the compound is acetonitrile.

5 Results

The profile of the proton NMR is presented in Figure 18. The 2D NMR profiles of HMQC and HMBC are shown in Figures 19 and 20, respectively.

Table 5.1 summarizes the 2D NMR chemical shift data and the assignment of functional groups derived from these data. Based on these data and analysis, the structure of compound Y (Y3) is assigned as shown below.

Figure 17: Structure of Compound Y



The chemical name of compound Y is: 3-O-[β-D-galactopyranosyl(1→2)]-α-L-arabinofuranosyl(1→3)-β-D-glucuronopyranosyl-21,22-O-diangeloyl-3β, 15α, 16α, 21β, 22α, 28-hexahydroxyolean-12-ene.

Table 5.1. ¹³C and ¹H NMR Data for Compound Y (in Pyridine-d₅)^a

Position	C	H	Key HMBC correlations
1	38.7	0.83, 1.40	C-3, C-5, C-9
2	26.4	1.81, 2.14	–
3	89.6	3.25, 1H, dd, 12.0/4.0 Hz	C-23, C-24, GlcA C-1'
4	39.4	–	–
5	55.3	0.78	–
6	18.5	1.55, 1.59	C-8, C-10
7	36.5	2.00, 2.10	C-5, C-9
8	41.2	–	–
9	47.0	3.06	C-7, C-8, C-12, C-14, C-26
10	37.2	–	–
11	23.7	1.74, 1.89	–
12	125.2	5.49, 1H, br s	C-9, C-11, C-14, C-18
13	143.4	–	–
14	47.5	–	–
15	67.3	4.21	C-8, C-27
16	73.6	4.45	C-14, C-15, C-18
17	48.3	–	–
18	40.8	3.07	C-12, C-13, C-14, C-16, C-19, C-20, C-28,
19	46.8	1.41, 1.69	–
20	36.2	–	–
21	79.3	6.71, 1H, d, 10 Hz	C-20, C-22, C-29, C-30, 21-O-Ang C-1'''
22	73.5	6.32, 1H, d, 10 Hz	C-16, C-17, C-21, C-28, 22-O-Ang C-1'''
23	27.7	1.26, 3H, s	C-3, C-4, C-5, C-24

24	16.5	1.16, 3H, s	C-3, C-4, C-5, C-23
25	16.0	0.81, 3H, s	C-1, C-5, C-9, C-10
26	17.3	0.99, 3H, s	C-7, C-8, C-9, C-14
27	21.0	1.85, 3H, s	C-8, C-13, C-14, C-15
28	62.9	3.50, 1H, d, 11.0 Hz, 3.76, 1H, d, 11.0 Hz,	C-16, C-17, C-18, C-22
29	29.2	1.09, 3H, s	C-19, C-20, C-21, C-30
30	20.0	1.32, 3H, s	C-19, C-20, C-21, C-29
GlcA			
1'	104.9	4.89, 1H, d, 7.8 Hz	C-3
2'	79.1	4.38	GlcA C-1', C-3', Gal C-1"
3'	86.1	4.20	GlcA C-2', C-4', Ara C-1'''
4'	71.5	4.42	GlcA C-3', C-5', C-6'
5'	78.0	4.52	GlcA C-4', C-6'
6'	171.9	–	–
Gal			
1"	104.6	5.32, 1H, d, 7.7 Hz	GlcA C-2'
2"	73.6	4.42	Gal C-1", C-3"
3"	74.9	4.10	Gal C-2"
4"	69.5	4.56	Gal C-2", C-3"
5"	76.4	3.94	Gal C-4", C-6"
6"	61.6	4.43, 4.52	Gal C-4", C-5"
Ara-f			
1'''	110.6	6.03. 1H, br s	GlcA C-3', Ara C-2''', C-4'''
2'''	83.4	4.94	Ara C-3'''
3'''	78.3	4.78	Ara C-2'''
4'''	85.2	4.82	Ara C-5'''
5'''	62.2	4.12, 4.28	Ara C-3'''
21-O-Ang			
1''''	167.7	–	–

2 ^{'''}	129.6	–	–
3 ^{'''}	137.2	5.96, 1H, dq, 7.0/1.5 Hz	Ang C-1 ^{'''} , C-4 ^{'''} , C-5 ^{'''}
4 ^{'''}	15.5	2.10, 3H, dq, 7.0/1.5 Hz	Ang C-2 ^{'''} , C-3 ^{'''}
5 ^{'''}	20.8	2.00, 3H, s	Ang C-1 ^{'''} , C-2 ^{'''} , C-3 ^{'''}
22-O-Ang			
1 ^{'''}	167.9	–	–
2 ^{'''}	129.8	–	–
3 ^{'''}	136.3	5.78, 1H, dq, 7.0/1.5 Hz	Ang C-1 ^{'''} , C-4 ^{'''} , C-5 ^{'''}
4 ^{'''}	15.5	1.93, 3H, dq, 7.0/1.5 Hz	Ang C-2 ^{'''} , C-3 ^{'''}
5 ^{'''}	20.5	1.74, 3H, s	Ang C-1 ^{'''} , C-2 ^{'''} , C-3 ^{'''}

^a The data were assigned based on HMQC and HMBC correlations.

The mass spectrum of compound Y as determined by MALDI-TOF and ESI-MS, i.e., see Figure 21, 22, indicates that the mass of compound Y is 1140.57 which agree with the theoretical mass of the compound Y.

Conclusion

The active compound Y isolated from extract of *Xanthoceras sorbifolia* is an oleanene triterpenoidal saponin with a trisaccharide chain attached at C-3 of the aglycone and two angeloyl groups acylated at C-21 and C-22. The formula of Y is C₅₇H₈₈O₂₃, and the chemical name of Compound Y is: 3-O-[β-D-galactopyranosyl(1→2)]-α-L-arabinofuranosyl(1→3)-β-D-glucuronopyranosyl-21,22-O-diangeloyl-3β, 15α, 16α, 21β, 22α, 28-hexahydroxyolean-12-ene.

Experiment 6: Determination of the Chemical Structure of Compound Y1 of Xanthoceras Sorbifolia Extract

Methods

- 5 The method for NMR and MS analysis for compound Y1 is similar to the method described in Experiment 5.

Results

- 10 The spectrum of the H-NMR is presented in Figure 24. The 2D NMR spectra of HMQC, HMBC and COSY are shown in Figures 25, 26 and 27, respectively. Table 6.1 summarizes the chemical shift data and the assignment of functional groups derived from these data.

Table 6.1. ¹³C and ¹H NMR Data for Compound Y1 (in Pyridine-d₅)

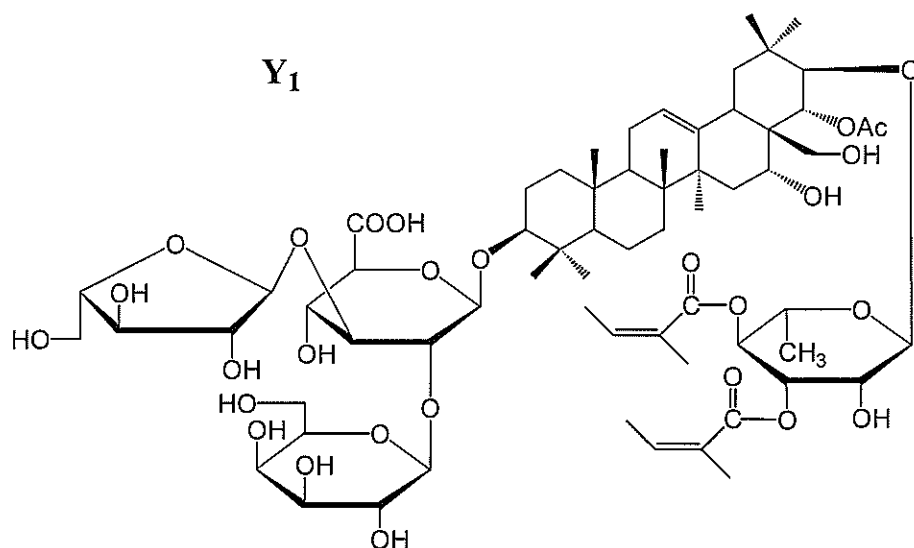
Position	C	H
1	38.6	0.85, 1.33
2	26.3	1.86, 2.10
3	89.7	3.25, 1H, dd
4	39.5	–
5	55.5	0.75
6	18.3	1.40, 1.43
7	33.1	1.20, 1.50
8	40.0	–
9	46.7	1.69
10	36.5	–
11	22.5	2.30
12	123.6	5.36, 1H, br s
13	143.5	–
14	41.8	–
15	34.7	1.53, 1.73

16	68.5	4.45
17	48.2	–
18	39.9	3.04
19	47.6	1.30, 3.05
20	36.7	–
21	85.3	5.05, 1H, d
22	73.8	6.17, 1H, d
23	27.7	1.29, 3H, s
24	16.5	1.16, 3H, s
25	15.5	0.81, 3H, s
26	17.1	0.82, 3H, s
27	20.6	1.83, 3H, s
28	63.7	3.42, 1H, d, 3.60, 1H, d
29	29.9	1.42, 3H, s
30	19.9	1.37, 3H, s
GlcA		
1	105.0	4.88, 1H, d
2	79.0	4.37
3	86.0	4.20
4	71.6	4.43
5	78.0	4.50
6	171.8	–
Gal		
1	104.5	5.31, 1H, d
2	73.5	4.43
3	74.9	4.10
4	69.5	4.57
5	76.3	3.95
6	61.1	4.44, 4.53
Ara-f		

1	110.9	6.04, 1H, br s
2	83.3	4.95
3	78.3	4.78
4	85.2	4.82
5	62.0	4.13, 4.31
21-O-Rha		
1	105.1	4.92, 1H, d
2	70.5	4.25
3	74.0	5.59
4	71.5	5.70
5	68.5	3.89
6	17.6	1.18, 3H, d
Rh-3-Ang		
1	167.2	–
2	127.9	–
3	138.7	5.92, 1H, q
4	15.7	2.02, 3H, d
5	20.6	1.92, 3H, s
Rh-4-Ang		
1	167.2	–
2	128.0	–
3	137.9	5.87, 1H, q
4	15.5	1.96, 3H, d
5	19.8	1.85, 3H, s
22-O-Ac		
1	171.4	–
2	21.8	2.31, 3H, s

Based on these data and analysis, the structure of compound Y1 is assigned and shown below.

Figure 23. Structure of Y1



The chemical name of Y1 is: 3-O-[β -D-galactopyranosyl(1 $\rightarrow$ 2)]- α -L-arabinofuranosyl(1 $\rightarrow$ 3)- β -D-glucuronopyranosyl-21-O-(3,4-diangeloyl)- α -L-rhamnopyranosyl-22-O-acetyl-3 β ,16 α , 21 β , 22 α , 28-pentahydroxyolean-12-ene.

Conclusion

Compound Y1 isolated from extract of *Xanthoceras sorbifolia* is a bisdesmosidic polyhydroxyoleanene triterpenoidal saponin with a trisaccharide chain at C-3 of the backbone and a monosaccharide moiety at C-21 where two angeloyl groups were acylated at C-3 and C-4 position. The formula of Y1 is $C_{65}H_{100}O_{27}$,

Experiment 7: Determination of the Chemical Structure of Compound Y2 of *Xanthoceras Sorbifolia* Extract.

Methods

The method for NMR and MS analysis for compound Y2 is similar to the method described in Experiment 5.

Results

The 1D and 2 D NMR spectra of H-NMR, C-13 NMR, HMQC, HMBC and (TOCSY) and MS (MALDI-TOF) of Y2 are showed in Figures 29-34. Table 7.1 summarizes the 1D and 2D NMR chemical shift data and the assignment of functional groups derived from these data.

Table 7.1: ¹³C and ¹H NMR data for Y2 (in Pyridine-d₅)^a

Position	C	H
1	38.4	0.83, 1.36
2	26.4	1.89, 2.25
3	91.3	3.39, 1H, m
4	43.4	–
5	56.7	0.87, 1H, d, 12.0 Hz
6	18.6	1.31, 1.57
7	36.3	1.97, 2.12
8	40.7	–
9	46.7	1.63
10	36.6	–
11	23.9	1.69, 1.89
12	125.1	5.48, 1H, br s
13	143.4	–
14	47.5	–
15	67.1	4.18, 1H, d, 4.1 Hz
16	73.2	4.43
17	48.1	–
18	41.4	3.06
19	46.6	1.40, 3.08
20	36.1	–
21	78.3	6.69, 1H, d, 10.2 Hz

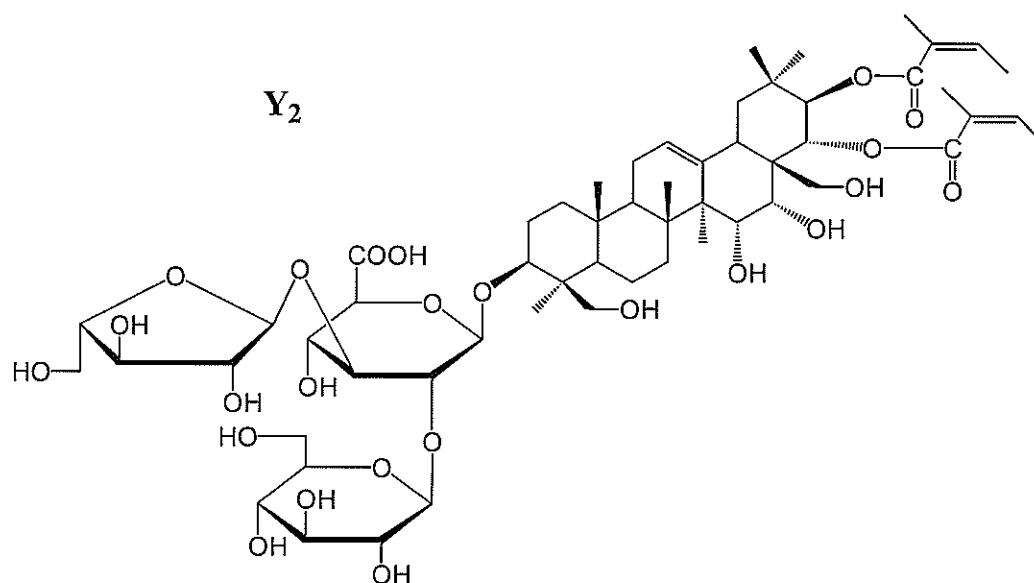
22	73.1	6.30, 1H, d, 10.2 Hz
23	22.0	1.29, 3H, s
24	62.9	3.28, 1H, d, 11.2 Hz; 4.32
25	15.6	0.64, 3H, s
26	17.1	0.94, 3H, s
27	20.8	1.84, 3H, s
28	63.1	3.48, 3.72 (each, 1H, d, 10.6 Hz)
29	29.3	1.09, 3H, s
30	20.0	1.32, 3H, s
3-O-GlcA		
1	104.5	4.87, 1H, d, 7.2 Hz
2	78.6	4.31
3	86.5	4.23
4	71.6	4.45
5	77.4	4.53
6	171.9	
Glc		
1	103.7	5.48, 1H, d, 7.8 Hz
2	75.3	4.02
3	78.0	4.31
4	69.3	4.52
5	78.2	3.62
6	61.5	4.33, 4.50
Ara		
1	110.1	6.05, 1H, br s
2	83.5	4.97
3	77.8	4.74
4	85.0	4.84
5	62.2	4.18, 4.33

21-O-ang		
1	167.5	–
2	128.7	–
3	137.2	5.95, 1H, dd, 14.4/7.2 Hz
4	16.7	2.08, 3H, d, 7.2 Hz
5	20.6	2.00, 3H, s
22-O-ang		
1	167.9	–
2	128.9	–
3	136.3	5.76, 1H, dd, 14.4/7.2 Hz
4	15.6	1.95, 3H, dd, 7.2 Hz
5	20.4	1.74, 3H, s

^a The data were assigned based on COSY, HMQC and HMBC correlations.

Conclusion

Based on these data and analysis, the compound Y2 isolated from extract of Xanthoceras sorbifolia is an oleanene triterpenoidal saponin with a trisaccharide chain attached at C-3 of the aglycone and two angeloyl groups acylated at C-21 and C-22. The chemical structure of Y2 is shown below. See also Figure 28.



The formula of Y2 is C₅₇H₈₈O₂₄, and the chemical name of Compound Y2 is: 3-O-[[β-D-glucopyranosyl-(1→2)]-α-L-arabinofuranosyl(1→3)-β-D-glucuronopyranosyl-21,22-O-diangeloyl-3β, 15α, 16α, 21β, 22α, 24β, 28-heptahydroxyolean-12-ene.

5

Experiment 7B. Chemical structure analysis of Y4

Results of Y4 analysis

The profile of the proton NMR of Y4 is presented in Figure 58. The profiles of 2D NMR (HMQC) of Y4 is presented in Figure 59.

10

Experiment 8. Purification of the Inhibition Components Y8-Y10 in the Xanthoceras Sorbifolia Extract

(A) Fractionation of Xanthoceras Sorbifolia extracts components with FPLC

Methods

The methods for this experiment are similar to the methods described in Experiment 4 Section (A) and (B).

Results

20

The elution profile shows 4-5 broad fractions. See Figure 9. These fractions were analyzed with HPLC. FPLC fractions 63, 64 and 65 are further separated on 45% isocratic analysis, 4-5 major components were separated (Figure 12). These fractions were assigned designations Y8, Y9 and Y10. These fractions were collected. Re-chromatography of the compound Y8, Y9 and Y10 showed a single peak in HPLC with a C18 reverse phase column. See Figure 13.

25

(B) Inhibition analysis with MTT assay.

Inhibition analysis of purified compounds was determined with the MTT assay. Results indicate that compound Y8, Y9 and Y10 has activity against ovarian cancer cells (OCAR-3) with IC₅₀ values of 3, 4 and 1.5 ug/ml, respectively. See Figure 4.

30

Experiment 9. Determination of the Chemical Structure of Compound Y8 of Xanthoceras Sorbifolia Extract

5 Methods

The method for NMR and MS analysis for compound Y8 is similar to the method described in Experiment 5.

Results

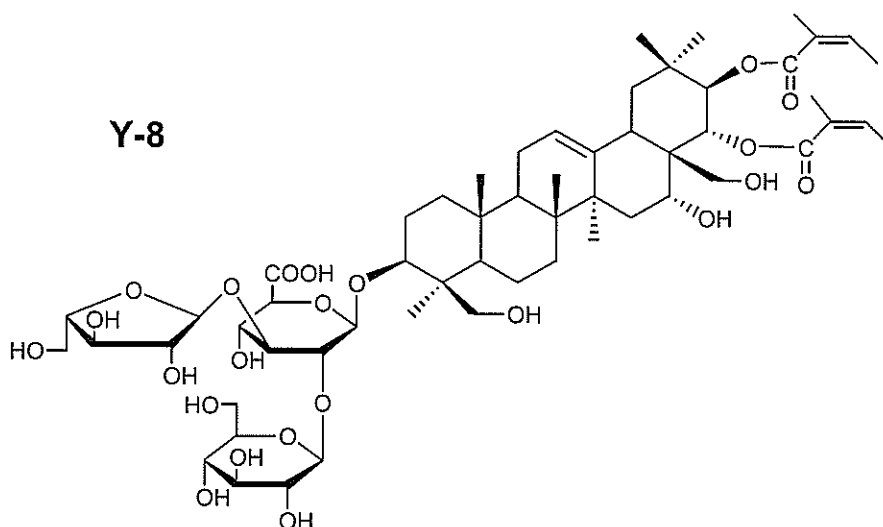
- 10 The spectral profiles of the H-NMR, C13-NMR 2D NMR (HMQC) of compound Y8 are presented in Figures 36-38.

Based on these data and analysis, the compound Y8 isolated from extract of Xanthoceras sorbifolia is an oleanene triterpenoidal saponin with a trisaccharide chain attached at C-3 of the aglycone and two angeloyl groups acylated at C-21 and C-22.

15

The formula of compound Y8 is $C_{57}H_{88}O_{23}$, and the chemical name of Y8 is: 3-O-[β -glucopyranosyl (1 $\rightarrow$ 2)]- α -arabinofuranosyl (1 $\rightarrow$ 3)- β -glucuronopyranosyl-21, 22-O-diangeloyl-3 β , 16 α , 21 β , 22 α , 24 β , 28-hexahydroxyolean-12-ene.

- 20 The chemical structure of compound Y8 is presented in the following figure. See a/so Figure 35.



Experiment 10. Determination of the Chemical Structure of Compound Y9 of Xanthoceras Sorbifolia Extract

5 Methods

The method for NMR and MS analysis for compound Y9 is similar to the method described in Experiment 5.

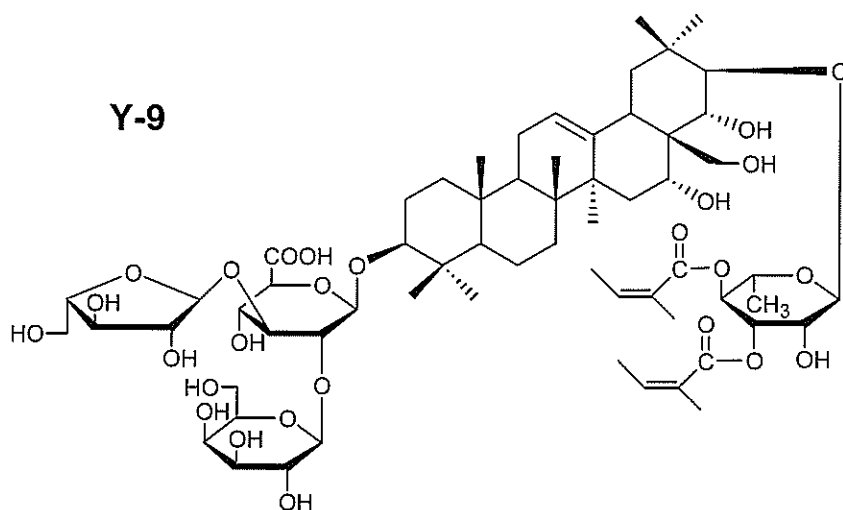
Results

- 10 The spectral profiles of the H-NMR, 2D NMR, i.e., HMQC and HMBC, of Y9 are shown in Figures 40-42.

Based on these data and analysis, compound Y9 isolated from extract of Xanthoceras sorbifolia is a bisdesmosidic polyhydroxyoleanene triterpenoidal saponin with a
15 trisaccharide chain at C-3 of the backbone and a monosaccharide moiety at C-21 where two angeloyl groups were acylated at C-3 and C-4 position.

The formula of compound Y9 is $C_{63}H_{98}O_{26}$, and the chemical name of Y9 is: 3-O-[β -galactopyranosyl (1 $\rightarrow$ 2)]- α -arabinofuranosyl (1 $\rightarrow$ 3)- β -glucuronopyranosyl-21-O-(3,4-
20 diangeloyl)- α -rhamnopyranosyl-3 β , 16 α , 21 β , 22 α , 28-pentahydroxyolean-12-ene.

The chemical structure of Compound Y9 is presented in the following figure. See *also* Figure 39.



Experiment 11. Determination of the Chemical Structure of Compound Y10 of *Xanthoceras Sorbifolia* Extract

5

Methods

The method for NMR and MS analysis for compound Y10 is similar to the method described in Experiment 5.

10 Results

The profile of the H-NMR, C13-NMR and 2D NMR (HMQC) are shown in Figures 44-46.

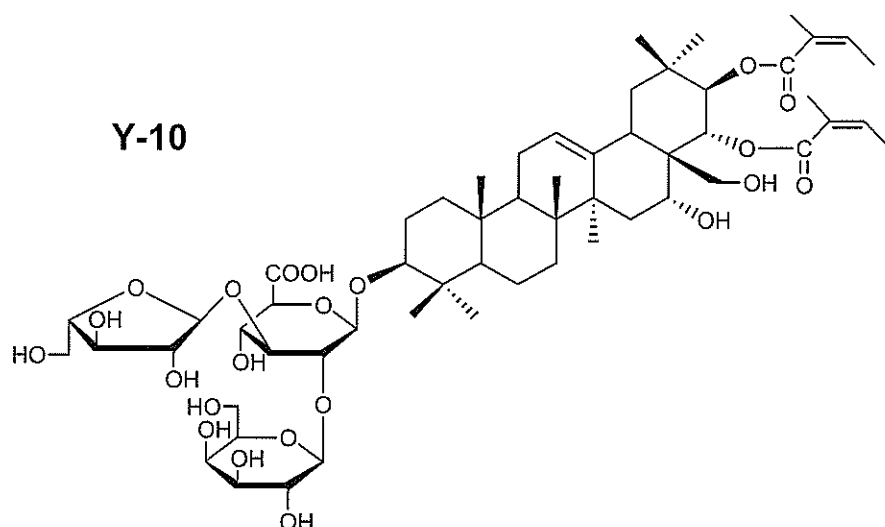
Based on these data and analysis, compound Y10 isolated from extract of *Xanthoceras sorbifolia* is an oleanene triterpenoid saponin with a trisaccharide chain attached at C-3 of the aglycone and two angeloyl groups acylated at C-21 and C-22.

15

The formula of compound Y10 is $C_{57}H_{87}O_{22}$, and the chemical name of Y10 is: 3-O-[β -galactopyranosyl (1 $\rightarrow$ 2)]- α -arabinofuranosyl (1 $\rightarrow$ 3)- β -glucuronopyranosyl-21, 22-O-diangeloyl-3 β , 16 α , 21 β , 22 α , 28-pentahydroxyolean-12-ene.

20

The chemical structure of Compound Y10 is presented in the following figure. See also Figure 43.



Experiment 12. Purification of Component R from *Xanthoceras Sorbifolia* Extract

5 (A) Purification of *Xanthoceras Sorbifolia* extracts components with FPLC and HPLC

Methods

The methods used are similar to the methods described in Experiment 4, section (A) and (B) except a 30% acetonitrile isocratic elution was used in HPLC for isolation of the Compound R.

Results

Fraction No. 39-41 from gradient elution of FPLC were pooled and further purified with an open ODS-C18 column with isocratic 30% acetonitrile elution. Six identifiable fractions in two groups were collected. Fractions 6-13 were further characterized with HPLC.

These fractions were further separated into 4-5 components with the 30% acetonitrile isocratic elution in a DeltaPak column. The fraction designated herein as "R1", is the major component. See Figure 60A. The pure R1 was subsequently collected from the column elution. See Figure 60B.

(B) Appearance and solubility

The pure R1 appears as an amorphous white powder, soluble in aqueous alcohol, i.e., methanol or ethanol, 50% acetonitrile and 100% pyridine.

5

(C) Determination of the chemical structure of R1

Methods

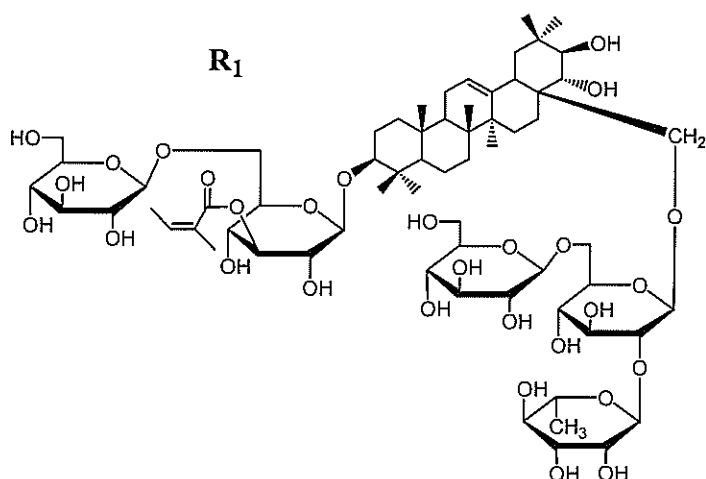
The NMR and MS Analysis of R1 is similar to the method described in Experiment 5.

10

Results

The NMR spectra of pure R1 is presented in Figures 48-52. Based on chemical shift analysis, compound R1 isolated from extract of *Xanthoceras sorbifolia* is a triterpenoid saponins with five sugars and one angeloyl group attached to the sugar moiety. The chemical structure of R1 is shown in following figure. See also Figure 47.

15



The formula of Compound R1 is $\text{C}_{65}\text{H}_{106}\text{O}_{29}$, and the chemical name of R1 is: 3-O-[angeloyl-(1 $\rightarrow$ 3)- β -D-glucopyranosyl-(1 $\rightarrow$ 6)]- β -D-glucopyranosyl-28-O-[α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-glucopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranosyl-3 β , 21 β , 22 α , 28-tetrahydroxyolean-12-ene.

20

Experiment 13: Purification of Component-O from Xanthoceras Sorbifolia extract

(A) Fractionation of Xanthoceras Sorbifolia extracts components with FPLC and HPLC

5

Methods

The methods used are similar to the methods described in Experiment 4, section (A) and (B) except a 20% acetonitrile isocratic elution was used in HPLC for isolation of the Compound O.

10

Results

Fractions obtained from FPLC were analyzed with HPLC. By comparison with the profiles of the original sample, a specific component, in this case fraction O, was identified (#28-30). Fraction O was collected for further purification. Sixteen identifiable HPLC fractions were observed in the elution profiles. See Figure 61. Fractions 28, 34 and 54 were further purified. See Figures 62-63. These purified components are named as compound O28, O34 and O54, respectively.

15

(B) Appearance and solubility

20

The purified compound O23 and O34 are light yellow amorphous powder, soluble in aqueous alcohol, i.e., methanol, ethanol, 50% acetonitrile and 100% pyridine. The purified compound O54 is a white amorphous powder, soluble in aqueous alcohol, i.e., methanol, ethanol, 50% acetonitrile and 100% pyridine.

25

(C) Structure analysis of Compound O54

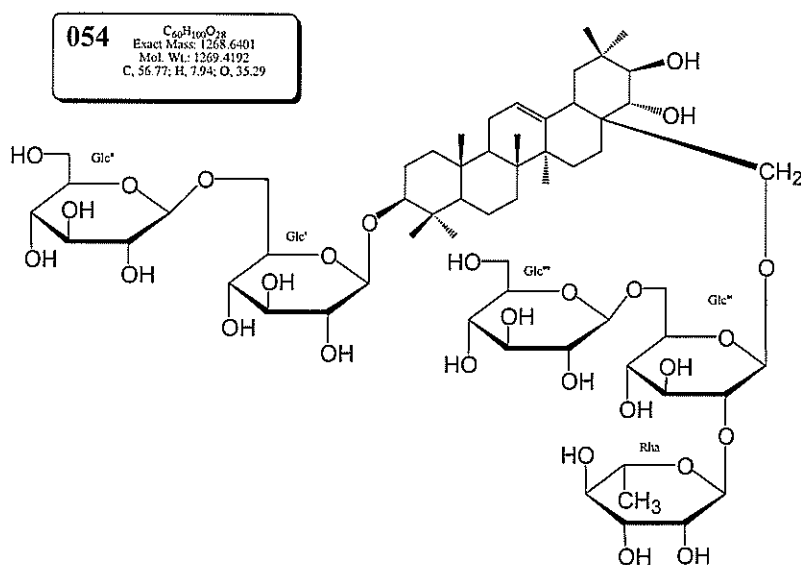
Methods

The NMR and MS analysis of O54 is similar to the method described in Experiment 5.

30

Results

The NMR spectra of compound O54 is presented in Figures 54-56. Based on the chemical shift analysis, compound O54 isolated from extract of *Xanthoceras sorbifolia* is a bisdesmosidic polyhydroxyoleanene triterpenoidal glycoside with a disaccharide chain [β -D-glucopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside] affixed to C-3 and a trisaccharide chain [α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-glucopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranosyl ester] attached to C-28. The chemical structure of compound O54 is presented in the following figure. See also Figure 53.



The formula of compound O54 is $C_{60}H_{100}O_{28}$, and the chemical name of O54 is: 3-O- β -D-glucopyranosyl-(1 $\rightarrow$ 6)]- β -D-glucopyranosyl-28-O-[α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-glucopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranosyl-3 β , 21 β , 22 α , 28-tetrahydroxyolean-12-ene.

Although the present invention has been described in detail with particular reference to preferred embodiments thereof, it should be understood that the invention is capable of other different embodiments, and its details are capable of modifications in various obvious aspects. As is readily apparent to those skilled in the art, variations and modifications can be affected while remaining within the spirit and scope of the invention. Accordingly, the foregoing disclosure, description, and figures are for

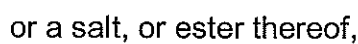
illustrative purpose only, and do not in any way limit the invention which is defined only by the claims.

REFERENCES

- 5 1. Carmichael, J., DeGraff, W.G., Gazdar, A.F., Minna, J.D. and Mitchell, J.B.: Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. *Cancer Res.* 47:936-942 (1987).
2. Chen, Q. 1995. Methods of study on pharmacology of Chinese medicines. Press of People's Public Health, Beijing. p892.
- 10 3. Huang, Zh. Sh., Liu, M. P., Chen, Ch. Zh. 1997. Study on effects of Yangshou Dan on improving learning and retention. *Chinese Journal of combination of Chinese and west medicine*, 9(17): 553.
4. Zhang, Y., Zhang, H. Y., Li, W. P. 1995. Study on effects of Anjifu on improving intelligence, *Chinese Bulletin of Pharmacology*, 11(3): 233.
- 15 5. Yang, J., Wang, J., Feng, P. A. 2000. Study on effects of Naokkangtai capsule on improving learning and retention in mice, *New Chinese Medicine and Clinical Pharmacology*, 1(11): 29.
6. Yang, J., Wang, J., Zhang, J. Ch. 2000. Study on effects of Crude saponins of peonies on improving learning and retention in mice, *Chinese journal of Pharmacology*, 2(16): 46.
- 20 7. Xia, W. J., Jin, M. W., Zhang, L. 2000. Study on treatment of senile dementia caused by angio-aging with Didang tang, *Pharmacology and Clinical of Chinese Medicines*, 16 (4).
8. Bian, H. M., Yu, J. Z., Gong, J. N. 2000. Study on effects of Tongmai Yizhi capsule on improving learning and retention in mice, *Pharmacology and Clinical of Chinese Medicines*, 16 (5): 40.
- 25 9. Wei, X. L., Zhang, Y. X. 2000. Study of animal model for studying senile dementia, *Chinese journal of Pharmacology*, 8(16): 372.
10. Bureau of Medicinal Police, Department of Public Health. Guide line for study of effect of medicines for treatment of nervous system diseases, in *Guidebook of study of new medicine*. p45.
- 30 11. Zhang, D. Sh., Zhang, J. T. 2000. Effects of crude Ginseng saponins on improving impairment induced by B- peptide, *Chinese journal of Pharmacology*, 8(16): 22.

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1. An isolated compound of formula (1):



R₁, R₂ represents angeloyl group or sugar moiety with diangeloyl groups;

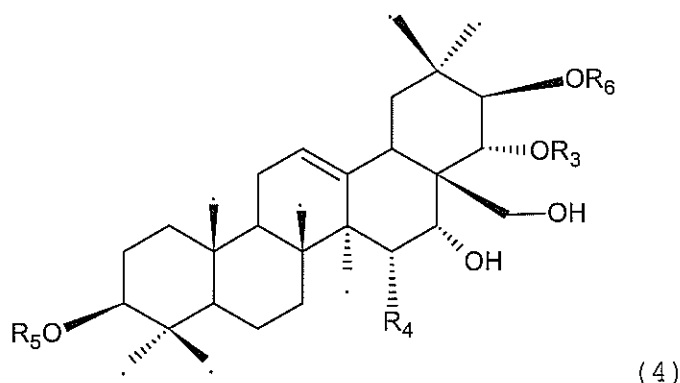
R₄ represents CH₃ or CH₂OH; and

R₅ represents D-glucose or D-Galactose.

R6 represents H or Ac

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8. An isolated compound selected from a compound of formula (4):



, a salt, or ester thereof,
wherein:

R₆ represents sugar moiety with diangeloyl groups in trans-position

R₃ represents Ac or H;

R₄ represents H or OH; and

R₅ represents sugar moiety or sugar chain, wherein at least one sugar is selected from the group consisting of: D-glucose, D-galactose, L-rhamnose, L-arabinose, D-xylose, alduronic acid, D-glucuronic acid and D-galacturonic acid.

9. A composition consisting essentially of an amount of the compound of claims 1 effective for inhibiting ovarian cancer cell growth.
10. The composition for inhibiting ovarian cancer cell growth, consisting essentially of an amount of the compound of claims 1 further comprising a pharmaceutically suitable carrier.

11. A composition consisting essentially of an amount of the compound of claim 9 effective for inhibiting ovarian cancer cell growth.
12. The composition for inhibiting ovarian cancer cell growth, consisting essentially of an amount of the compound of claim 9 further comprising a pharmaceutically suitable carrier.
13. A composition consisting essentially of an amount of the compound of claim 1 effective for inhibiting bladder, bone, leukocyte, liver, prostate, breast or brain cancer cells growth.
14. A composition consisting essentially of an amount of the compound of claim 9 effective for inhibiting bladder, bone, leukocyte, liver, prostate, breast or brain cancer cells growth.

